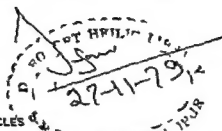


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SUDDEN INFANT DEATH IN COPENHAGEN 1956-1971

II Social Factors and Morbidity

FIN BIERING SØRENSEN TORBEN JØRGENSEN and JØRGEN HILDEN

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ABSTRACT Biering Sørensen F Jørgensen T and Hilden J (Municipal Agency of Infant Health Visitors Copenhagen and Institute of Human Genetics University of Copenhagen Copenhagen Denmark) Sudden infant death in Copenhagen 1956-71 II Social factors and morbidity *Acta Paediatr Scand* 68 1 1979.—131 cases of the Sudden Infant Death Syndrome (SIDS) among infants born in the Municipality of Copenhagen during 1956-71 were analysed on the basis of data collected prospectively by the infant health visitors and abstracted from police reports. Compared with controls a significantly larger number of SIDS infants had been born out of wedlock, were living only with their mother, had parents in a less secure occupation, lived in more crowded, poor-quality dwellings and districts. The home standard was lower among the SIDS families, including a lower standard of infant care, a higher percentage of not keeping the appointments with the infant health visitors, and a lower mental capacity in the mothers. There was no difference with respect to the mother's physical capacity. More congenital malformations and more cases of asphyxia were found among the SIDS infants, and a significantly larger number of the SIDS infants had been admitted to hospital, with a tendency to being kept there longer. It is concluded that a relationship exists between poor social conditions, increased morbidity, and SIDS. At the same time it is pointed out that during recent years the differences between cases and controls as regards certain of the social parameters studied have decreased.

KEY WORDS Sudden infant death, socioeconomic factors, infant care, morbidity.

The Sudden Infant Death Syndrome (SIDS) and its relationship to certain social parameters have been known for many years (1-8, 17). The present paper is intended to shed more light on these aspects as well as the morbidity of the SIDS victims, using prospectively collected data. It forms part of a larger epidemiological study of SIDS in the Municipality of Copenhagen (3, 10, 11).

MATERIAL AND METHODS

SIDS is defined here as: The sudden death at home of an infant between one week and one year, which is unexpected by history and in which a thorough post mortem examination fails to demonstrate an adequate cause for death (3).

The material comprises all 139 SIDS victims born in the Municipality of Copenhagen during the period 1956-71 (0.9 SIDS cases per 1000 live births). Age at death ranged from 10 to 359 days (median 170 days, mean 113 days) (3). Police reports were available for all these cases.

In 131 of the cases (44 females and 87 males) prospectively collected data were obtained from the case records filed by the infant health visitors in the Municipality of Copenhagen (3). Each of these 131 SIDS cases was matched by the aid of the infant health visitors' files with four controls living at home and with a few exceptions of the same sex and birth date (3). Among the SIDS cases (hereinafter called cases) there were 26 (19.8%) prematures (birth weight ≤ 2500 g) and among the 574 controls 16 (3.1%).

Data concerning symptoms or illness during the last 4 weeks before death are derived from the police reports. All other results, i.e. information concerning social factors and morbidity, are from data collected by the health visitors.

During the study period the population of Copenhagen Municipality fell from 746 034 in 1956 to 625 678 in 1971. The number of live born infants was 9817 in 1956 and remained at this level until 1963, when it rose to 10 568, where it remained until 1966. It then dropped to 9690 in 1967 and fell steadily thereafter to 7751 in 1971.

In order to detect temporal trends, the time under investigation was divided into four year periods as follows, unless otherwise stated: 1956-59, 1960-63, 1964-67, and 1968-71. It should be noted that the third four year period is characterized by a high birth rate, reflecting the fact

Table 1 *Percentage (and number) of cases and controls born in and out of wedlock (left) and living with their parents or only with their mother (right)*

	Born		Living with	
	In wedlock	Out of wedlock	Both parents	Only the mother
Cases	76.3 (100)	23.7 (31)	95.9 (107)	17.1 (22)
Controls	93.1 (488)	6.9 (36)	95.4 (500)	4.6 (24)
χ^2 (d.f.)	30.39 (1)		22.73 (1)	
p value	<0.0001		<0.0001	

that the approx. 20-year-olds born during the high birth rate years around 1945 were then having their firstborns. This factor was taken into consideration in evaluating temporal trends.

The chi-square and Mann-Whitney rank-sum tests were used in the statistical analysis with 5% as significance level.

RESULTS

Civil status

A significantly larger number of cases than of controls were born out of wedlock and a larger number of cases were living with their mother alone (Table 1). The number of cases as well as controls living with both parents exceeded that of those born in wedlock due to the considerable number of unmarried couples living together. The values in the table have been subdivided by sex but there was no difference. Division into prematures and matures also showed no difference in the trend. Sub-division of the material into the named four-year periods revealed nothing apart from the general tendency to fewer infants born in wedlock.

Parents' occupation

The parents' occupation is recorded by the infant health visitors at the first visit, the mother's as that up to the possible childbirth leave.

Table 2 shows no significant difference in mother's occupation between cases and controls. When regarding only mothers who go out to work there are significantly more salaried employees (including civil servants)

among the control mothers, whereas the case mothers were more often wage earners ($\chi^2 = 3.88$, d.f. = 1, $p = 0.049$). Sub-division into the four-year periods showed no definite differences in the pattern except for the increased tendency to go out to work.

As to mothers living alone with their infants (22 and 24 in the last column of Table 1) there is no difference with respect to occupation. In both groups only 20% did not go out to work.

Table 3 sets out the father's occupation when the baby was living with both parents. There is a significantly larger number of salaried employees in the control group and more wage earners in the SIDS group. From 1956-59 to 1968-71 there was a tendency for this difference between the case and control group to diminish; the percentage of salaried em-

Table 2 *Percentage (and number) of cases and controls whose mothers did not go out to work, were salaried employees, or were wage earners*

Mother's occupation	Cases	Controls
Did not go out to work	69.4 (84)	66.7 (126)
Salaried employees incl. civil servants	19.0 (23)	26.4 (129)
Wage earners	11.6 (14)	7.0 (34)
χ^2 (d.f.)	4.85 (2)	
p value	0.088	
Other occupations	(10)	(30)
Unknown	(0)	(5)
Did not go out to work 1956-59	87.5 (28)	88.3 (113)
Did not go out to work 1968-71	37.9 (11)	35.3 (41)

Table 3 Percentage (and number) of cases and controls whose fathers were salaried employees or wage earners provided the infant was living with both parents

Father's occupation	Cases	Controls
Salaried employees incl civil servants	20.4 (19)	38.1 (158)
Wage earners	79.6 (74)	61.9 (253)
χ^2 (d.f.)	9.65 (1)	
p value	0.0019	
Other occupations	(8)	(71)
Unknown	(6)	(14)

employees in the control group remaining practically unchanged (about 38%) while in the SIDS group it rose from 17% to 28%.

Neither the data underlying Table 2 nor those of Table 3 showed any sex difference.

In Tables 2 and 3 the term 'other occupations' indicates self employed and liberal. They were excluded from the present calculations partly because these occupations are very inhomogeneous and partly because the groups are relatively small.

Salaried employees in general may be said to have a more secure occupational status than wage earners. This is primarily because the former have one to three months' notice whereas the wage earners often can be sacked at day's notice. Besides, the members of the salaried employees' group generally have better education and possibilities of earning more. When considering the occupation of cohabiting parents jointly, it is possible therefore to set up more or less socially secure occupational combinations. It was found that 22.6% of the cases and 41.4% of the controls had parents in a socially more secure occupational combination characterized by the father being a salaried employee and the mother also a salaried employee or not going out to work. Correspondingly 77.4% of the cases and 58.6% of the controls had parents in a socially less secure occupational combination, the father being a wage earner and the mother a wage earner or not going out to work. This

aspect differed significantly for the cases and controls ($\chi^2=9.41$, d.f. = 1, $p=0.0021$).

Housing

In Table 4 the districts of the infant health visitors in the Municipality of Copenhagen are divided into above average, average and below average. This classification was made by the head of the Municipal Agency of Infant Health Visitors by informally estimating the housing and the social composition and standard of the districts, but not knowing the figures of the present study. It should be mentioned here that the residential quarters of the well-to-do are almost entirely outside the Municipality of Copenhagen. It is apparent from the table that a significantly larger number of cases than of controls lived in the below average districts. There was no sex difference and there was no definite trend to a change in the difference through the years.

Table 5 shows that the person/room ratio was significantly higher in the case group than in the control group. There was no sex difference. Apart from a general reduction of this ratio from 1956-59 to 1968-71, there was no definite change in the pattern evident from Table 5.

The quality of the dwelling (Table 6) was as

Table 4 Percentage (and number) of cases and controls who lived in different districts in the Municipality of Copenhagen, classified as above average, average and below average

Above average: Østerbro, Brønshøj, Vanløse and Valby.
Average: City, Amager, North West, Sydhavn and the neighbouring part of Vesterbro.
Below average: Islands Brygge, Christianshavn, Nørrebro and the remaining part of Vesterbro.

Districts	Cases	Controls
Above average	28.1 (36)	38.8 (202)
Average	37.8 (47)	36.9 (197)
Below average	39.1 (50)	24.4 (127)
χ^2 (d.f.)	11.75 (2)	
p value	0.0077	
Unknown	(3)	(3)

Table 5 Percentage (and number) of cases and controls by number of persons per room

Persons per room (P/R)	Cases	Controls
(P/R) ≤ 1	5.7 (7)	15.6 (78)
1 < (P/R) ≤ 2	74.6 (91)	71.3 (356)
2 < (P/R)	19.7 (24)	13.0 (65)
χ^2 (d.f.)	10.16 (2)	
p value	0.0062	
Unknown	(9)	(27)

essed by the infant health visitors. The classification into above average, average and below average was based on whether the dwelling was dark, humid, without a separate kitchen, bathroom or toilet, plus its lay out and maintenance on the whole. On the other hand, this evaluation paid no regard to the number of rooms and persons. It applies to the data in Tables 6-9 that all are based upon a personal estimate and that the manner of estimating has changed somewhat in the course of the study period. Since, however, they were collected in a prospective manner for cases and controls born at the same time, a bias has probably been avoided. In Table 6 there are significantly more cases than controls in the poorer dwellings. There was no sex difference and no change in the pattern from 1956-59 to 1968-71.

Home standard

The economy of the home (Table 7) was estimated by the health visitors as being above average, average or below average. This was not particularly on the basis of the actual earnings by the family, but according to the way in which these earnings were spent, for instance whether the family could cover expenses beyond bare necessities. There was a significantly larger number of cases than of controls in the below average group. Of all the social variables studied, the economy of the home was that which had the strongest association

Table 6 Percentage (and number) of cases and controls by quality of dwelling as classified by the infant health visitor

Quality of dwelling	Cases	Controls
Above average	17.7 (72)	31.4 (161)
Average	56.5 (70)	60.7 (311)
Below average	25.8 (32)	7.8 (40)
χ^2 (d.f.)	35.37 (2)	
p value	< 0.0001	
Unknown	(7)	(17)

with SIDS. This association remained unchanged throughout the study period.

Table 8 lists the infant health visitors' estimate as to the mother's so-called mental and physical capacity. The mental capacity, which was significantly poorer for cases than for controls, was estimated on the basis of the mother's attitude to the infant as well as her ability and determination to follow the guidance given by the infant health visitor. When the material was divided into four year periods, a significant difference was found only during that from 1956-59 ($\chi^2 = 7.73$, d.f. = 1, $p = 0.005$), the difference between cases and controls decreasing as time went by (the excess percentage of above average control mothers over the above average case mothers being, during the successive four year periods, 26%, 17%, 12% and 4%). The mother's physical capacity, in which there was no difference between

Table 7 Percentage (and number) of cases and controls by economy of the home as classified by the infant health visitor

Economy of the home	Cases	Controls
Above average	5.5 (5)	10.9 (51)
Average	69.2 (63)	84.6 (396)
Below average	25.3 (73)	4.5 (71)
χ^2 (d.f.)	46.24 (7)	
p value	< 0.0001	
Unknown	(40)	(56)

Table 8 Percentage (and number) of cases and controls by mother's mental and physical capacity as classified by the infant health visitor

	Mother's mental capacity		Mother's physical capacity	
	Cases	Controls	Cases	Controls
Above average	39.6 (36)	56.0 (267)	60.8 (59)	63.2 (301)
Average	57.1 (57)	47.3 (207)	37.1 (36)	36.3 (173)
Below average	3.3 (3)	1.7 (8)	2.1 (2)	0.4 (2)
χ^2 (d.f.)		7.63 (1)		0.11 (1)
p value		0.0058		0.74
Unknown	(40)	(47)	(34)	(48)

cases and controls was estimated according to whether the mother had sufficient strength to look after her home and child/children

Infant care

The care of the infant including cleanliness was also estimated by the infant health visitors and classified as above average, average and below average. In cases where it was classified as being below average, there were serious objections. Table 9 shows significantly better care of controls than of cases. This difference was also reduced as time went by. Regrettably, the criterion for classifying above average has altered in the course of the study period, but an impression of the trend is gained by using χ^2 as a measure of case-control difference: the findings for the successive four-year periods were 0.24, 0.27, 0.13 and 0.09.

The percentage of appointments not kept (Table 10) shows that the case parents were

less conscientious than the control parents in keeping the appointments. In this respect there were no definite changes in the trend through the years.

Malformations and morbidity

Four cases and six controls had congenital malformations. Within the SIDS group there was one case of syndactyly, one of polydactyly, one of a deformed foot and bilateral testicular hydrocele, and lastly one case of intestinal eversion and malrotation. The malformations in the controls were foot deformities in four, harelip and cleft palate in one and ureteric stricture in one. Apart from actual malformations, two SIDS infants had hernia, one umbilical and one inguinal. Within the control group one infant had an umbilical hernia. Lastly, it should be mentioned that among the SIDS cases neonatal asphyxia had been recorded by the midwife in three, two of whom were premature (birth weights 1400 and

Table 9 Percentage (and number) of cases and controls by infant care as classified by the infant health visitor

Infant care	Cases	Controls
Above average	54.3 (63)	69.8 (359)
Average	44.0 (51)	30.2 (155)
Below average	1.7 (1)	(0)
χ^2 (d.f.)		9.64 (1)
p value		0.0019
Unknown	(15)	(10)

Table 10 Percentage (and number) of cases and controls by mother's keeping appointments with infant health visitor

Appointments not kept (ANK)	Cases	Controls
(ANK) $\leq 10\%$	55.1 (70)	72.9 (380)
$10\% < \text{(ANK)} \leq 70\%$	18.1 (23)	15.7 (87)
$70\% < \text{(ANK)}$	26.8 (34)	11.3 (59)
χ^2 (d.f.)		22.09 (2)
p value		0.0001

Table 11 Percentage (and rate) of cases and controls hospitalized for their own disease during their lifetime and within the first year of life respectively

Also subdivided according to birth weight. For 29 cases and 100 controls it is unknown whether they have been hospitalized

	All infants	Matures (birth weight >2 500 g)	Prematures (birth weight ≤2 500 g)
Cases	33.3 (=34/102)	21.8 (=17/78)	70.8 (=17/24)
Controls	13.0 (=55/424)	12.9 (=53/411)	15.4 (=2/13)
χ^2 (d.f.)	22.82 (1)	3.54 (1)	8.28 (1)
p value	<0.0001	0.060	0.004

1500 g). In the control group there had been no instances of asphyxia.

To gain an impression of the morbidity of the SIDS infants we analysed from the health visitors' records how many cases had been in hospital because of a disease of their own (not their mother's) (Table 11). If more than 30 days had elapsed between the last visit by the infant health visitor and the infant's death and the infant had not previously been admitted to hospital, it was considered unknown whether the infant had been in hospital. By way of comparison we used the number of controls who had been in hospital during the first year of life. Although the average life span of the cases was only 133 days and the observation period for the controls was 365 days Table 11

shows that significantly more cases than controls had been admitted to hospital. This tendency applied to matures as well as pretermatures but it will be noted that the greater part of the difference is explained by admissions for prematurity (*vide infra*).

In the attempt to assess the gravity of the hospital admissions we analysed for those admitted (34 cases and 55 controls) the number of days in hospital for each infant. For matures the mean stay was 26.9 and 23.2 days for cases and controls respectively. For pretermatures the corresponding values were 51.3 and 42.0 days. Thus the duration of the stay in hospital was about 20% longer for cases but this difference is not significant judging by the Mann-Whitney test.

Table 12 Percentage (and number) of 139 SIDS cases without or with symptoms or illness during the fourth to second week before death, the seventh to second day before death and the last day before death

During the period fourth to second week before death two SIDS cases had had two symptoms from the seventh to second day before death four had had two symptoms and on the last day three had had two symptoms

	Fourth to second week before death	Seventh to second day before death	Last day before death
No symptoms or illness	72.3 (81)	49.2 (62)	47.2 (59)
Respiratory symptoms or diseases	16.1 (18)	34.1 (43)	35.2 (44)
Otitis media	3.6 (4)	6.3 (8)	4.0 (5)
Gastrointestinal symptoms or diseases	4.5 (5)	10.3 (13)	8.8 (11)
Other symptoms or diseases	5.4 (6)	3.2 (4)	7.2 (9)
Unknown	(27)	(13)	(14)

Table 13 Percentage (and number) of 139 SIDS cases grouped according to month of death

For comparison a series of 51 infants who died of respiratory infections at the age of one week to one year have been similarly grouped (cf. text). The statistical tests indicate non-uniform distributions over the year.

	SIDS	Respiratory deaths
Cold months (DJFM)	4* 4 (59)	49 0 (25)
Warm months (JIAS)	5 2 (35)	9 8 (15)
Remaining months (AM+ON)	3* 4 (45)	41 7 (21)
χ^2 (d.f.)	6.27 (7)	13.18 (2)
p value	0.043	0.0014

The main hospital diagnoses were prematurity (13 cases, 1 control), otitis media (2 cases, 7 controls), respiratory diseases including pneumonia, bronchitis, acute laryngitis, influenza and whooping cough (2 cases, 10 controls), gastrointestinal conditions in particular dyspepsia and acute gastroenteritis (7 cases, 14 controls), and other diagnoses (7 cases, 20 controls). Lastly the diagnosis in 3 cases and 3 controls is unknown.

From the police reports we have extracted data concerning all 139 SIDS cases as regards symptoms or diseases antecedent to death (Table 12). During the last week rather more than half the SIDS infants had had symptoms, about 75% of which were respiratory symptoms or otitis media. On the other hand less than 30% had had symptoms during the second to fourth week before death.

The month of death. Table 13 is grouped into the four warmest, the four coldest and the four remaining months of the year. A significantly larger number of SIDS cases died during the cold months. From the study period 1956 to 1971 an analysis was made of deaths from respiratory infections without preceding illness at the age of one week to one year. In this category too deaths occurred significantly more often during the cold months (for further description of this material cf. (10)). With respect to month at death there was

no sex difference within the groups. Among the 131 SIDS cases for whom the health visitor's record includes information about birth weight there was also no difference in this respect between maturers and prematures.

DISCUSSION

Socioeconomic factors

Like most previous studies (1, 2, 4-6, 8, 9, 12-15, 17, 18) ours has shown that the SIDS infants were from an underprivileged environment: a large number being born out of wedlock and living only with their mothers, having parents with not so secure occupations, living in the poorer parts of town, in more crowded and poor quality dwellings (Tables 1-6).

In addition the data collected by the infant health visitors gave an impression of the home standard. The parents of the SIDS cases were not particularly skilled in dealing with their earnings (Table 7) and the mothers' mental capacity was below that in the control group (Table 8). An estimate of maternal intelligence and effectiveness has been published previously (16) but in a broader group of infant deaths. The results pointed into the same direction. We also found that infant care (Table 9) was of lower standard and that the health visitors more often visited the SIDS families in vain, finding no one at home at the appointed time (Table 10).

On the other hand there was no difference between the case and control groups with regard to the mothers' physical capacity (Table 8). This we interpret to the effect that the psychosocial family background—in directly reflected by the variables just mentioned—is more likely to trigger the occurrence of SIDS in the family than is insufficient maternal strength for the purely physical care of the baby. At the same time it must be pointed out that the difference between cases and controls with respect to maternal mental capacity and the infant's care seems to have decreased during recent years.

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Also subdivided according to birth weight For 29 cases and 100 controls it is unknown whether they have been hospitalized

	All infants	Matures (birth weight >2 500 g)	Prematures (birth weight ≤2 500 g)
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To gain an impression of the morbidity of the SIDS infants, we analysed from the health visitors' records how many cases had been in hospital because of a disease of their own (not their mother's) (Table 11). If more than 30 days had elapsed between the last visit by the infant health visitor and the infant's death, and the infant had not previously been admitted to hospital, it was considered unknown whether the infant had been in hospital. By way of comparison, we used the number of controls who had been in hospital during the first year of life. Although the average life span of the cases was only 133 days and the observation period for the controls was 365 days, Table 11

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During the period fourth to second week before death two SIDS cases had had two symptoms, from the seventh to second day before death four had had two symptoms, and on the last day three had had two symptoms

	Fourth to second week before death	Seventh to second day before death	Last day before death
No symptoms or illness	72.3 (81)	49.2 (62)	47.2 (59)
Respiratory symptoms or diseases	16.1 (18)	34.1 (43)	35.2 (44)
Otitis media	3.6 (4)	6.3 (8)	4.0 (5)
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Remaining months (AM+ON)	32.4 (45)	41.2 (71)
χ^2 (d.f.)	6.7 (*)	13.18 (2)
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The main hospital diagnoses were prematurity (13 cases, 1 control), otitis media (2 cases, 7 controls), respiratory diseases including pneumonia, bronchitis, acute laryngitis, influenza and whooping cough (2 cases, 10 controls), gastrointestinal conditions in particular dyspepsia and acute gastroenteritis (7 cases, 14 controls), and other diagnoses (7 cases, 20 controls). Lastly the diagnosis in 3 cases and 3 controls is unknown.

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This tendency towards obliterating differences in the social parameters including the father's occupation, will be further elucidated in a subsequent paper (10)

It may be emphasized that to this general impression of a poorer social environment in the SIDS group there are several exceptions, in our material too viz infants from environments which are generally considered among the best

Morbidity

Like Fedrick (7) we found more malformations in the SIDS group than among the controls. We also found as did Næye et al (15) more cases of asphyxia which can of course be related to the lower average birth weight in the SIDS group. All considered, it indicates a less favourable start in life. However the values are too small to form the basis of definite conclusions.

Evaluating the dead infants morbidity through life is very difficult as it is hard to find a relevant control group. To this end we chose to analyse the number of infants who had been admitted to hospital (Table 11) and the duration of the stay in hospital. For want of a better basis of comparison we compared the SIDS infants until their death with the controls during their first year of life. And even so we could demonstrate a tendency to more hospital admissions and a longer duration of stay in hospital for the SIDS cases, the matures as well as the prematures. This must be taken to mean that the infants in the SIDS group were more and more seriously ill than the infants of the control group or at least were thought to be more in need of being admitted to hospital and of being kept there longer e.g. for social reasons.

Froggatt et al (8) and Fedrick (7) have also compared the morbidity of SIDS cases with that in a control group. Like us they found more hospital admissions among the SIDS infants than among the controls. In two materials without a control group (2, 4) the frequency of admissions for SIDS cases has been

reported to be only 6.5% (11/170) and 10.9% (14/128). The former authors even state that the SIDS infants tended to be remarkably healthy.

As regards the causes of admissions to hospital our material like that of others is too small to form the basis of conclusions. However it is worth mentioning that as compared with the findings of Froggatt et al (8) and of Fedrick (7) respiratory disorders made up only a few of the admission diagnoses among our SIDS cases and corresponded to those in the controls during the first year of life.

With respect to symptoms during the weeks prior to death there is also no good control group. It may be expected (6) that when questioned about an infant who has suddenly died without any signs of serious disease the parents will try to attribute a certain importance even to the slightest symptoms. On the other hand the parents of living controls or of controls who have died of a known cause are more apt to overlook symptoms the infant has recently had. In evaluating the accumulation of symptoms among the cases during the last weeks before death (Table 12) over-reporting of recent and under-reporting of somewhat earlier symptoms may play a role.

That respiratory symptoms are the most common ones immediately before death (Table 12) is in close accordance with the previous findings (2, 4, 6) that between 44 and 68% of the SIDS cases had had respiratory disorders within the last two weeks before death. But the great majority of these had been colds or sniffles. This corresponds to the larger number of SIDS deaths during the cold months of the year (Table 13) (2, 4, 7-9) in which there is also an accumulation of deaths from respiratory infections. Actually the distribution of the SIDS group looks like a less accentuated version of that of respiratory deaths. This is what might be expected if part of the SIDS deaths were in fact undetectable fatal respiratory infections.

CONCLUSION

Our results show that as a whole the SIDS infants were from socioeconomically under privileged environments and were more often ill than their controls. At the same time however we have observed that the association between SIDS and certain of the less favourable social parameters has disappeared during recent years. It is important therefore to investigate in future studies whether the social differences between cases and controls are decreasing and whether other differences have taken over. In a subsequent paper (10) this aspect will be studied more closely.

Neither our studies nor previous ones have demonstrated a distinct pattern let alone specific causes of SIDS deaths. Therefore it must be assumed that several underlying conditions exist. Maybe in predisposed infants various factors can trigger death, an apparently trivial infection being one such possible factor (Tables 12 and 13). This accords entirely with the hypothesis that the SIDS age range represents a period of enhanced physiological vulnerability (8).

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REFERENCES

- 1 Beckwith J B. The sudden infant death syndrome. *Curr Probl Pediatr* 3:3 1973.
- 2 Bergman A II, Ray C G, Pomeroy M A, Wahl P W & Beckwith J B. Studies of the sudden infant death syndrome in King County, Washington III. Epidemiology. *Pediatrics* 49:860 1972.
- 3 Biering Sørensen F, Jørgensen T & Hilden J. Sudden infant death in Copenhagen 1946-1971. I. Infant feeding. *Acta Paediatr Scand* 67:179 1978.
- 4 Borhani N O, Rooney P A & Kraus J F. Post

- neonatal sudden unexplained death in a California community. *Calif Med* 118:12 1973.
- 5 Cameron A H & Asher P. Cot deaths in Birmingham 1958-61. *Med Sci Law* 5:187 1965.
- 6 Carpenter E G & Shaddick C W. Role of infection, suffocation and bottle feeding in cot death. An analysis of some factors in the histories of 110 cases and their controls. *Br J Prev Soc Med* 19:1 1965.
- 7 Fedrick J. Sudden unexpected death in infants in the Oxford Record Linkage Area. Details of pregnancy, delivery and abnormality in the infant. *Br J Prev Soc Med* 28:164 1974.
- 8 Froggatt P, Lynas M A & MacKenzie G. Epidemiology of sudden unexpected death in infants (cot death) in Northern Ireland. *Br J Prev Soc Med* 25:119 1971.
- 9 Hostetk J. Sudden infant death syndrome in Czechoslovakia. Epidemiologic aspects. In A B Bergman J B Beckwith & C G Ray (eds). *Sudden infant death syndrome*. University of Washington Press, Seattle and London 1970. p. 55.
- 10 Jørgensen T, Biering Sørensen F & Hilden J. Sudden infant death in Copenhagen 1946-1971. III. Perinatal and perimortal factors. *Acta Paediatr Scand* 68:11 1979.
- 11 Jørgensen T, Biering Sørensen F & Hilden J. Sudden infant death in Copenhagen 1946-1971. IV. Infant development. To be published.
- 12 Kraus J F, Steele R, Thompson M E & de Grosbois P. Further epidemiologic observations on sudden unexpected death in infancy in Ontario. *Can J Public Health* 62:210 1971.
- 13 Kraus J F & Borhani N O. Post neonatal sudden unexplained death in California. A cohort study. *Am J Epidemiol* 95:497 1972.
- 14 McWeeny P M & Emery J L. Unexpected post neonatal deaths (cot deaths) due to recognizable disease. *Arch Dis Child* 50:191 1975.
- 15 Naeye I L, Ladis B & Drage J S. Sudden infant death syndrome. A prospective study. *Am J Dis Child* 130:1207 1976.
- 16 Richards I D G & McIntosh H T. Confidential inquiry into 276 consecutive infant deaths. *Arch Dis Child* 47:697 1972.
- 17 Valdes Dapena M A. Sudden and unexpected death in infancy. A review of the world literature 1954-1966. *Pediatrics* 39:123 1967.
- 18 Valdes Dapena M, Birle L J, McGovern J A, McGillen J F & Colwell F H. Sudden unexpected death in infancy. A statistical analysis of certain socioeconomic factors. *J Pediatr* 73:387 1968.

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This tendency towards obliterating differences in the social parameters including the father's occupation will be further elucidated in a subsequent paper (10)

It may be emphasized that to this general impression of a poorer social environment in the SIDS group there are several exceptions in our material too viz infants from environments which are generally considered among the best

Morbidity

Like Fedrick (7), we found more malformations in the SIDS group than among the controls. We also found as did Næye et al (15) more cases of asphyxia which can of course be related to the lower average birth weight in the SIDS group. All considered it indicates a less favourable start in life. However the values are too small to form the basis of definite conclusions.

Evaluating the dead infants morbidity through life is very difficult as it is hard to find a relevant control group. To this end we chose to analyse the number of infants who had been admitted to hospital (Table 11) and the duration of the stay in hospital. For want of a better basis of comparison we compared the SIDS infants until their death with the controls during their first year of life. And even so we could demonstrate a tendency to more hospital admissions and a longer duration of stay in hospital for the SIDS cases, the matures as well as the prematures. This must be taken to mean that the infants in the SIDS group were more and more seriously ill than the infants of the control group or at least were thought to be more in need of being admitted to hospital and of being kept there longer e.g. for social reasons.

Froggatt et al (8) and Fedrick (7) have also compared the morbidity of SIDS cases with that in a control group. Like us they found more hospital admissions among the SIDS infants than among the controls. In two materials without a control group (2, 4) the fre-

quency of admissions for SIDS cases has been reported to be only 6.5% (11/170) and 10.9% (14/128). The former authors even state that the SIDS infants tended to be remarkably healthy.

As regards the causes of admissions to hospital our material like that of others is too small to form the basis of conclusions. However it is worth mentioning that as compared with the findings of Froggatt et al (8) and of Fedrick (7) respiratory disorders made up only a few of the admission diagnoses among our SIDS cases and corresponded to those in the controls during the first year of life.

With respect to symptoms during the weeks prior to death there is also no good control group. It may be expected (6) that when questioned about an infant who has suddenly died without any signs of serious disease the parents will try to attribute a certain importance even to the slightest symptoms. On the other hand the parents of living controls or of controls who have died of a known cause are more apt to overlook symptoms the infant has recently had. In evaluating the accumulation of symptoms among the cases during the last weeks before death (Table 12) over-reporting of recent and under-reporting of somewhat earlier symptoms may play a role.

That respiratory symptoms are the most common ones immediately before death (Table 12) is in close accordance with the previous findings (2, 4, 6) that between 44 and 68% of the SIDS cases had had respiratory disorders within the last two weeks before death. But the great majority of these had been colds or sniffles. This corresponds to the larger number of SIDS deaths during the cold months of the year (Table 13) (2, 4, 7-9) in which there is also an accumulation of deaths from respiratory infections. Actually the distribution of the SIDS group looks like a less accentuated version of that of respiratory deaths. This is what might be expected if part of the SIDS deaths were in fact undetectable fatal respiratory infections.

SUDDEN INFANT DEATH IN COPENHAGEN 1956-1971

III Perinatal and Perimortal Factors

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ABSTRACT Jørgensen T Biering Sørensen F and Hilden J (Municipal Agency of Infant Health Visitors Copenhagen and Institute of Human Genetics University of Copenhagen Copenhagen Denmark) Sudden infant death in Copenhagen 1956-1971 III Perinatal and perimortal factors *Acta Paediatr Scand* 68 11 1979.—139 cases of the Sudden Infant Death Syndrome (SIDS) among infants born in the Municipality of Copenhagen during the period 1956-1971 were analysed on the basis of data collected from police reports and (for 131 cases) from the infant health visitors' records. In the SIDS group there was a greater male preponderance than among others dying in the same age range. Compared with the living controls the SIDS mothers had attended less prenatal examinations, more often delivered their babies at home, the SIDS parents were younger, and yet the SIDS infants were less often firstborns. There was no difference with respect to history of abortions, maternal state of health during pregnancy, or events at delivery. The age at death for the SIDS infants is of a distribution similar to that for fatal respiratory infections. Prematures died later than maturates, but this difference is not statistically significant. It is concluded that perinatal factors and SIDS are correlated, but owing to changes in predisposing factors and decreasing differences between cases and controls in recent years as well as interdependence of the factors, it seems doubtful whether the incidence of SIDS can be reduced by alleviating the above mentioned unfavourable factors.

KEY WORDS Sudden infant death, social conditions, prenatal care, abortions, birth order, respiratory tract infections.

The Sudden Infant Death Syndrome (SIDS) and its correlation to various perinatal and perimortal factors have been elucidated in several previous studies (2, 8, 9, 17, 18, 22, 23). After the theory of external suffocation as a cause of SIDS has been gradually abandoned in the middle of this century (2, 25), the conditions under which the infant is found dead have not attracted major interest. Instead, the age at death and the numerous factors relating to gestation and labour have given food for further research and theorizing (2, 8, 9, 18, 24, 27).

On the basis of data collected prospectively by the infant health visitors, police report data and extracts of death certificates from the National Health Service, we have tried by the present study to gain further and more de-

tailed information about these factors. It was endeavoured also to demonstrate the interdependence of these factors and their change in time, parallel with the findings in a previous study (6). In the light of our findings, the possibilities of reducing the incidence of SIDS are discussed.

The material forms part of a larger epidemiological study of SIDS in the Municipality of Copenhagen (5, 6, 13).

MATERIAL AND METHODS

For the delimitation of the material we adopted the following modification of the SIDS definition proposed by Beckwith at the Second International Conference on the Causes of SIDS in 1969 (3). The sudden death at home of infants between one week and one year, which is unexpected by history and in which a thorough post mortem examination fails to demonstrate an adequate cause of death.

Table 2 *Percentage and (number) of cases and controls born at home in maternity clinics and in hospitals*

Total divided into birth order one and more than one

Place of birth	Total		Birth order one		Birth order more than one	
	Cases	Controls	Cases	Controls	Cases	Controls
Home	21 (26)	10 (47)	5 (7)	4 (8)	7 (23)	15 (39)
Maternity clinic	19 (24)	77 (127)	13 (5)	27 (56)	22 (19)	27 (71)
Hospital	60 (75)	63 (301)	82 (31)	69 (145)	51 (44)	59 (156)
χ^2 (d.f.)	12.12 (*)		3.79 ()		6.66 (*)	
p-value	0.002		0.19		0.016	
Unknown	(6)	(49)	(5)	(28)	(1)	(21)

A few studies (16-25) have shown a female and the remainder (2-7, 20, 22-25) a male SIDS preponderance. Froggatt et al. (11) found 58.8% boys in the SIDS group against 53.2% among all other deaths within the same age range.

Prenatal care

In Denmark nine prenatal visits to the doctor and/or midwife are recommended. We consider eight or more visits to be satisfactory attendance.

As seen from Table 1 a significantly larger number of SIDS than control mothers did not give satisfactory attendance, and this is further illustrated by the mean number of visits. A particularly large number of SIDS mothers paid only 1-3 visits. There was no sex difference.

Division of the material. Division by place of birth shows the same difference as described above for mothers having their babies in hospital or maternity clinics. Only mothers having their babies at home show no significant difference in attendance (Table 1). Thus what constitutes the major part of the difference in the Total portion of the table is the large number of control mothers with satisfactory prenatal attendance and having their babies in hospitals or maternity clinics.

We also divided the material by birth order viz. firstborns and others, but this showed no

difference from the pattern in the Total of Table 1. There was not unexpectedly a tendency to fewer prenatal visits at a high birth order among case as well as control mothers.

Maternal health during pregnancy and course of delivery

In 19 (15%) of the SIDS mothers and 70 (14%) of the control mothers the state of maternal health during pregnancy was not considered good. The disorders in question were preeclampsia, nausea and vomiting (emesis), anaemia and various trivial diseases such as mild infections. As regards mild infections there were five incidents among the 70 control mothers but none among the 19 SIDS mothers.

In 9 (7%) of the SIDS mothers and 27 (5%) of the control mothers the delivery had not been normal. Either it had been operative—by forceps, vacuum extractor or caesarean section—or else the foetus had presented by the breech. There was no sex difference.

Place of birth

A distinction will be made between three places of birth viz. at home, in a maternity clinic and in hospital. In Denmark a maternity clinic is a maternity institution having certain technical facilities and a fixed staff of midwives. A doctor is not constantly present but

Table 1 Percentage and (number) of case and control mothers visiting the doctor and/or midwife less than eight times for prenatal care

Total divided into deliveries at home and in maternity institutions For 11 cases and 28 controls attendance was unknown

No of visits	Total		Born at home		Born in maternity clinic/hospital	
	Cases	Controls	Cases	Controls	Cases	Controls
<8	48 (57)	33 (164)	44 (11)	43 (19)	48 (46)	32 (145)
≥8	52 (63)	67 (332)	56 (14)	57 (25)	52 (49)	68 (107)
χ^2 (d f)	8.14 (1)		0.034 (1)		8.52 (1)	
p value	0.0044		0.85		0.0035	
Mean	7.04	8.33	7.00	7.57	7.05	8.41

A further description of the selection was given in the first paper of the present series (5).

Our material comprises all 139 SIDS cases born in the Municipality of Copenhagen during the period 1956 to 1971. For all cases we have information from the police reports which are mandatory in such cases. Furthermore the prospectively collected data from the municipal infant health visitors' records were available for 131 of the cases (44 girls and 87 boys) (5).

Each of these 131 SIDS cases was matched with four controls from the infant health visitors' files. These controls were living at home and with a few exceptions they were of the same sex and born on the same day (5).

From the National Health Service we obtained information about the sex, age at death and cause of death for all infants born in the Municipality of Copenhagen during the period 1956-1971 and dying at the age of one week to one year.

Data concerning perinatal factors such as circumstances at death and the time when found dead are from the police reports whereas the perinatal data are derived from the health visitors' records. Let it be mentioned that with the exception of maternal health during pregnancy, the perinatal data are also included in the midwife's record sent to the infant health visitor prior to her first visit to the home.

It must be pointed out that the so called perinatal factors to be elucidated here are often of a social nature and their distinction from the social factors previously reported (6) is therefore arbitrary. Indeed this will be apparent from our conclusions.

In assessing time trends we shall refer unless otherwise stated to the following four year periods: 1956-59, 1960-63, 1964-67 and 1968-71 which are further characterized in the preceding paper (6).

The statistical tests used were the chi square, Mann-Whitney rank sum test and combinatorial reasoning. Significance level 5%.

RESULTS

Incidence

The incidence of SIDS was 0.92 cases per 1000 live born as stated in the first paper of

this series (5). At the same time it was pointed out that the incidence had remained constant through the period 1956 to 1971 while among whites in San Francisco (26) the incidence had fallen from 1.2 in 1961 to 0.7 in 1971.

Within the material of 131 SIDS cases there were 26 (19.8%) prematures (birth weight ≤ 2500 g) and among the 524 controls 16 (3.1%).

During the time under study 1956-71 the SIDS deaths made up 15.7% of all deaths in the age range concerned. This proportion has not been constant: the SIDS cases amounting to 12.6% (66/525) during the years 1956-63 and to 20.3% (73/360) during the years 1964-71. This significant change ($\chi^2=9.01$, d.f.=1, $p=0.0027$) reflects a fall in general infant mortality while the incidence of SIDS remained constant. In two previously published studies the SIDS cases have made up about one third of the deaths occurring from 1 week to 1 year (2) and from 4 weeks to 51 weeks (12).

Sex ratio

In the material of 139 SIDS cases 94 (68%) were boys and 45 (32%) girls. Among the remaining infants born in the Municipality of Copenhagen from 1956 to 1971 and dying at the age of one week to one year 419 (56%) were boys and 327 (44%) girls. The difference in the sex ratio between the SIDS cases and the others is statistically significant ($\chi^2=5.85$, d.f.=1, $p=0.016$).

Table 4 Percentage and (number) of case and control mothers whose age at delivery was ≤ 19 20-24 25-29 and ≥ 30 and whose civil status was unmarried and married

Age	Total		Mother unmarried		Mother married	
	Cases	Controls	Cases	Controls	Cases	Controls
≤ 19	11 (23)	7 (35)	7 (9)	11 (6)	14 (14)	6 (79)
20-24	56 (73)	43 (725)	58 (18)	46 (16)	56 (55)	43 (709)
25-29	11 (74)	31 (159)	6 (7)	23 (8)	27 (72)	31 (151)
≥ 30	8 (10)	19 (100)	6 (7)	11 (5)	8 (8)	70 (95)
χ^2 (d.f.)	31.39 (3)		5.37 (2)		18.66 (3)	
p-value	<0.0005		0.070		<0.0005	
Mean age	27.8	25.5	21.9	23.9	23.1	25.6
Unknown	(1)	(5)	(0)	(1)	(1)	(4)

week) are included but not previous abortions

According to Table 6 the SIDS cases were more rarely firstborns than the controls. There was no sex difference.

Division of the material. Matures and pre-matures showed the same tendency.

The pattern has altered in the course of time (Table 7). In 1956-59 there was a marked preponderance of SIDS cases of a birth order of two or more but in 1968-71 this feature had vanished.

Abortions

Abortion is defined here as provoked or spontaneous interruption of a pregnancy before the 28th week producing a dead foetus.

None of the women had a history of more

than four abortions. There was no significant case-control difference (Table 8). No sex difference.

Among the mothers in Table 8 with a history of abortions there is a tendency to multiple (i.e. 2-4) abortions in SIDS mothers. However, the difference is hardly significant ($n=114$, $\chi^2=3.83$, d.f.=1, $p=0.050$).

Lastly, we compared the total number of abortions with the total number of pregnancies. This revealed (Table 8) a high abortion rate in SIDS mothers but the difference is not significant ($n=1142$ pregnancies, $\chi^2=0.17$, d.f.=1, $p=0.68$).

Maternal age corrected for parity

The pattern showing a higher birth order for cases than for controls (Table 6) is seen also when the maternal is divided by maternal age

Table 5 Percentage and (number) of case and control fathers whose age at the birth of the children was ≤ 24 25-29 30-34 and ≥ 35

Father's age	Cases	Controls
≤ 24	45 (45)	8 (137)
25-29	31 (31)	37 (180)
30-34	1 (1)	70 (98)
≥ 35	1 (1)	14 (69)
χ^2 (d.f.)	11.5 (3)	
p-value	0.005 < p < 0.010	
Mean age	6.8	28.5
Unknown	(3)	(40)

Table 6 Percentage and (number) of cases and controls divided by birth order one two and three or more

Birth order	Cases	Controls
1	33 (43)	45 (237)
2	47 (54)	36 (187)
≥ 3	25 (33)	19 (100)
χ^2 (d.f.)	6.64 (2)	
p-value	0.036	
Unknown	(1)	(0)

Table 3 *Percentage and (number) of cases and controls born at home in maternity clinics and in hospitals for four periods of time*

Cases tested against controls for the periods 1956-63 and 1964-71 since otherwise the numbers would be too small

Place of birth	1956-59		1960-63		1964-67		1968-71	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Home	29 (9)	20 (24)	32 (9)	7 (7)	18 (7)	5 (7)	4 (1)	9 (9)
Maternity clinic	19 (6)	28 (33)	14 (4)	25 (25)	24 (9)	31 (47)	18 (5)	21 (22)
Hospital	52 (16)	53 (63)	54 (15)	68 (67)	58 (22)	65 (99)	79 (22)	70 (72)
χ^2 (d f)	9.14 (2)						3.13 (2)	
p value	0.010						0.21	

■ general practitioner or an obstetrician is routinely called so that only a few deliveries take place without a doctor being present

Table 2 shows that a significantly larger proportion of cases than of controls were born at home while there was ■ preponderance of controls born in maternity clinics. With respect to deliveries in hospital there was no major difference. There was no sex difference.

Division of the material Considering first borns and others (Table 2) the latter group follows the pattern from the Total of the table. Within the group of firstborns there were only a few births at home and in this respect there was no difference between cases and controls. At the same time there was a preponderance of hospital births for cases.

If the married and unmarried mothers are considered separately the married ones follow the pattern from the Total in Table 2 where as—not surprisingly—the unmarried mothers follow the pattern of the primiparae. Nearly all unmarried mothers were delivered in hospital (cases 94%, controls 81%).

A division into matures and prematures shows for both groups the same pattern as in the Total of Table 2.

In Table 3 the material is divided into four chronological periods. It will be seen that births at home have been declining among cases as well as controls. This is more striking for cases the preponderance of home deliveries in the SIDS group (Table 2) having disappeared in 1968-71. Besides the difference

between the proportion of cases and controls born in maternity clinics is decreasing.

The birth place distribution of the control group altered significantly with time (Table 3 $\chi^2=8.43$ d f = 2 $p=0.015$).

Age of parents

The SIDS mothers were significantly younger (Table 4) the pattern being the same for married and unmarried mothers. There was no sex difference.

Division of the material Matures showed the same pattern (maternal mean age 22.6 years in the SIDS group ($n=105$) and 25.6 years in the control group ($n=505$)) as in the Total of Table 4. This typical pattern was not present among the prematures the maternal mean age for SIDS cases being 23.7 years ($n=25$) and that for controls 23.1 years ($n=16$).

The difference in mean age between SIDS and control mothers diminished in the course of time (1956-59 4.3 years 1960-63 2.8 years 1964-67 2.4 years and 1968-71 1.5 years).

Paternal age (Table 5) showed the same pattern as maternal age. Only the fathers were about 3 years older. Fathers of unknown age were mainly those who were not living with the mothers. There was no age difference between the fathers of boys and girls.

Parity

In calculating the birth order previous still births (the birth of a dead foetus after the 28th

Table 4 Percentage and (number) of case and control mothers whose age at delivery was ≤ 19 20-24 25-29 and ≥ 30 and whose civil status was unmarried and married

Age	Total		Mother unmarried		Mother married	
	Cases	Controls	Cases	Controls	Cases	Controls
≤ 19	18 (23)	7 (35)	20 (9)	17 (6)	14 (14)	6 (79)
20-24	56 (73)	43 (7.5)	58 (18)	46 (16)	56 (55)	43 (709)
25-29	18 (24)	31 (159)	6 (7)	23 (8)	27 (27)	31 (151)
≥ 30	8 (10)	19 (100)	6 (7)	14 (5)	8 (8)	20 (95)
χ^2 (d.f.)	31.39 (3)		5.37 ()		18.66 (3)	
p-value	<0.0005		0.070		<0.0005	
Mean age	27.8	25.5	21.9	23.9	23.1	25.6
Unknown	(1)	(5)	(0)	(1)	(1)	(4)

week) are included but not previous abortions

According to Table 6 the SIDS cases were more rarely firstborns than the controls. There was no sex difference.

Division of the material. Matures and pre-matures showed the same tendency.

The pattern has altered in the course of time (Table 7). In 1956-59 there was a marked preponderance of SIDS cases of a birth order of two or more but in 1968-71 this feature had vanished.

Abortions

Abortion is defined here as provoked or spontaneous interruption of a pregnancy before the 28th week producing a dead foetus.

None of the women had a history of more

than four abortions. There was no significant case-control difference (Table 8). No sex difference.

Among the mothers in Table 8 with a history of abortions there is a tendency to multiple (i.e. 2-4) abortions in SIDS mothers. However, the difference is hardly significant ($n=114$, $\chi^2=3.83$, d.f.=1, $p=0.050$).

Lastly, we compared the total number of abortions with the total number of pregnancies. This revealed (Table 8) a high abortion rate in SIDS mothers but the difference is not significant ($n=142$ pregnancies, $\chi^2=0.17$, d.f.=1, $p=0.68$).

Maternal age corrected for parity

The pattern showing a higher birth order for cases than for controls (Table 6) is seen also when the material is divided by maternal age

Table 5 Percentage and (number) of case and control fathers whose age at the birth of the children was ≤ 24 25-29 30-34 and ≥ 35

Father's age	Cases	Controls
≤ 24	45 (45)	28 (137)
25-29	31 (31)	37 (180)
30-34	1 (1)	70 (98)
≥ 35	1 (1)	14 (69)
χ^2 (d.f.)	11.5 (3)	
p-value	0.005 < p < 0.010	
Mean age	6.8	28.5
Unknown	(31)	(40)

Table 6 Percentage and (number) of cases and controls divided by birth order one two and three or more

Birth order	Cases	Controls
1	33 (43)	45 (237)
2	47 (54)	36 (187)
≥ 3	25 (33)	19 (100)
χ^2 (d.f.)	6.64 (7)	
p-value	0.036	
Unknown	(1)	(0)

Table 7 Percentage and (number) of cases and controls whose birth order was one two or three or more divided into four periods of time

Birth order	1956-59		1960-63		1964-67		1968-71	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
1	13 (4)	38 (48)	31 (9)	40 (46)	38 (15)	55 (90)	48 (14)	45 (51)
2	65 (20)	43 (54)	28 (8)	32 (37)	43 (17)	31 (51)	31 (9)	38 (43)
≥3	23 (7)	19 (24)	41 (12)	28 (32)	20 (8)	14 (23)	21 (6)	18 (20)
χ^2 (d.f.)	7.46 (2)		2.12 (2)		3.91 (2)		0.45 (2)	
p value	0.024		0.35		0.14		0.79	

(Table 9), except in the group aged 30 and over.

For each of the birth orders the SIDS mothers are younger than the control mothers (Fig 1). On the basis of this curve it is tempting to assume that the second child follows soon upon the first in SIDS mothers.

By regarding the maternal mean age at the various birth orders in the control group as the age at which a woman normally has her first (second, third, ...) baby it is possible to elucidate the effect of maternal age corrected for parity. In this way the women may be divided into two groups: Group A consisting of mothers who were young compared with the number of children they had borne (i.e. one child and less than 23 years, two children and less than 26 years, three children and less than 29 years, and four or more children and less than 31 years) and group B comprising the others. Table 10 shows that a significant surplus of SIDS mothers were young for parity.

Table 8 Percentage and (number) of cases and controls whose mothers had a history of no one and 2-4 abortions

No. of abortions	Cases	Controls
None	82 (98)	81 (401)
1	12 (14)	16 (78)
2-4	7 (8)	2 (14)
χ^2 (d.f.)	5.06 (2)	
p value	0.080	
Total abortions	13.4	12.2
Total pregnancies	(33/246)	(109/896)
Unknown	(11)	(31)

If this principle is applied to the four periods separately (Table 11) the difference between SIDS cases and controls will be seen to be decreasing in time.

Infant's age at death

The three curves in Fig. 2 show the age at death (between one week and one year) for SIDS cases for all other infants born in the Municipality of Copenhagen in 1956-71 and for those of the latter who died of respiratory tract infections without pre-existing chronic disease. The diagnoses in the last mentioned group were: Acute pharyngitis, acute laryngitis and tracheitis, acute bronchitis, lobar pneumonia, bronchopneumonia, and empyema. These diagnoses are according to the WHO classification.

The curve for all non-SIDS deaths shows a marked accumulation of deaths within the first month and is obviously different from the

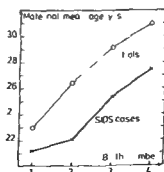


Fig. 1 Mean age of SIDS and control mothers at delivery plotted against birth order (first, second, third, fourth or more child).

Table 9 Percentage and (number) of cases and controls of birth order one two and three or more when maternal age at delivery was ≤ 19 20-24 25-29 and ≥ 30

Birth order	≤ 19		20-24		25-29		≥ 30	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
1	59 (13)	91 (37)	37 (73)	59 (133)	17 (4)	36 (57)	70 (2)	13 (13)
2	41 (9)	9 (3)	51 (37)	37 (71)	17 (4)	44 (70)	40 (4)	41 (41)
≥ 3	0 (0)	0 (0)	18 (13)	9 (71)	66 (16)	70 (37)	40 (4)	46 (46)
χ^2 (d.f.)	6.67 (1)		17.04 (7)		73.35 (7)		0.0009 (1)	
P-value	0.0099		0.0007		<0.000045		0.98	

other two curves. The SIDS curve has a low start in order to reach a peak at the age of 2-4 months. We dare not attach importance to the fact that this peak is cleft. The curve representing deaths from respiratory tract infections is rather similar to that of SIDS cases.

We did not observe any sex difference in any of the three materials with respect to age at death.

For the 139 SIDS cases the mean life span was 132 days. 30% had died before 3 months and 76% before 6 months of age.

The mean life span of infants succumbing to respiratory tract infections exceeded that of SIDS infants by only 16 days. The difference was in fact not significant according to the Mann-Whitney test ($t=0.98$, d.f. = 188, $0.30 < p < 0.40$).

The mean life span of the 26 premature SIDS cases was 152 days and that of the 105 mature SIDS cases 128 days. This difference is not significant according to the Mann-Whitney test ($t=1.54$, d.f. = 129, $0.10 < p < 0.20$).

Table 10 Percentage and (number) of cases and controls whose mothers were young for parity (A) and the remainder (B)

For further explanation see the text

	Cases	Controls
A	84 (108)	90 (258)
B	16 (11)	90 (61)
χ^2 (d.f.)	47.5 (1)	
P-value	<0.0001	

Circumstances at death

17 (13%) died while sleeping in the same bed as another person, the others while lying alone in a pram, carry cot, cradle, child's bed or adult bed. 16 of the 17 who died while sharing a bed with others were boys. This sex difference is significant ($n=134$, $\chi^2=4.59$, d.f. = 1, $p=0.032$).

79 (62%) were found with vomit or frothy fluids in the mouth and nostrils, commonly blood-tinged, staining the bedclothes. In this respect there was no sex difference.

41 (47%), 22 (25%) and 25 (28%) were found dead lying prone, supine and on their side respectively. A significantly larger number of boys were found lying supine, whereas

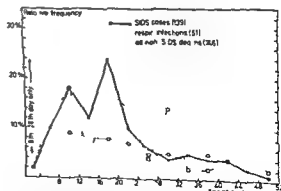


Fig. 2. Curves showing the age at death for SIDS cases, all other infants dying, and infants dying of respiratory tract infections without preceding chronic disease. All three curves represent infants born in the Municipality of Copenhagen dying during the age interval 1 week - 1 year. Numbers in brackets.

Table 11 The procedure of Table 10 applied to each of the four periods of time

	1946-59		1960-63		1964-67		1968-71	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
A	90 (28)	48 (60)	90 (26)	47 (54)	75 (30)	48 (78)	69 (70)	49 (56)
B	10 (3)	52 (66)	10 (3)	53 (61)	25 (10)	52 (86)	31 (9)	51 (58)
χ^2 (d.f.)	16.72 (1)		15.42 (1)		8.65 (1)		2.90 (1)	
p value	<0.0001		<0.0001		0.0033		0.089	

more girls were found lying on their side ($\chi^2 = 8.48$ d.f. = 2 $p = 0.014$)

In 35 cases it was recorded in the police reports that the infants had been found dead their face down or covered with a pillow or eiderdown. In this respect there was no sex difference.

The percentages in this section were calculated on the basis of how many police reports mentioned the factor concerned.

Hour when found dead

Fig. 3 illustrates the hour of the day or night when the infant was found dead. This was known for 90 boys and 40 girls. There is an accumulation in the morning comprising almost all the girls and the majority of the boys. In addition, a small proportion of the boys were found during the afternoon or evening. This sex difference is significant ($p = 0.022$) assessed as the probability that there is a series of 24 boys interrupted by only one girl if 90 boys and 40 girls are arranged randomly around the clock.

The interval between last seen alive and found dead averaged 4.1 hours (range 0-13 hours, median 4 hours, $n = 122$). No infant was observed at the moment of death.

DISCUSSION

Perinatal factors

The perinatal characteristics of which we found a preponderance in the SIDS group were: Less attendance for prenatal examinations (Table 1), young parents (Tables 4 and 5), birth order two and over (Table 6) and de-

livery at home (Table 2). This agrees with previous studies (1, 2, 8, 11, 12, 14, 15, 17, 18, 23, 25). In a Canadian study (14) however it is stated that the difference in maternal age at delivery was unimpressive and not statistically significant (80 cases and 80 controls) but the SIDS mothers' age at first delivery was lower than that of the control mothers. In a report from Australia (1) the parity for 36 cases was compared with that for all live born during the same period and there was little difference. No authors have discussed deliveries at home versus deliveries in institutions.

The increased risk of SIDS among mothers who are young for parity, which seems to be the natural interpretation of Table 11, supports the findings of Steele & Langworth (22) viz that the SIDS mothers were younger than the control mothers when cases and controls were matched according to parity. Since moreover a high parity is an added risk factor at a given maternal age (Table 9), age and parity appear

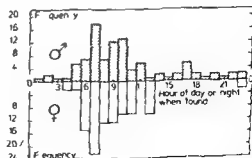


Fig. 3 Hour of day or night at which the SIDS cases were found dead, divided into boys (90 in all) and girls (40 in all). Midnight = 0 = 24 hrs. With one single exception no girls were found dead in the hours of the afternoon or evening.

to exert an opposed influence upon the risk of SIDS

Thus SIDS mothers are characterized by earlier or more frequent pregnancies but the proportion of these pregnancies ending in abortions is not demonstrably increased

It is difficult to make comparison with other studies as regards abortions as the definitions of abortions have differed or been lacking. Most authors report no difference between case and control mothers (8, 11, 14, 21). However, Naeye (17) found more abortions among SIDS mothers whereas Steele & Langworth (22) found more previous abortions among control mothers.

As regards the state of health during pregnancy and the course of delivery, our negative results correspond largely to previous findings (12, 14, 17, 18) but three publications (9, 17, 18) have reported a preponderance of various diseases during pregnancy among SIDS mothers.

For some of the parameters studied viz prenatal care (Table 1), place of birth (Table 2), maternal age (Table 4) and parity, we divided the Total in the tables into various sub-groups in order to be able to compare cases and controls that shared a characteristic of established importance. These sub-divisions were done in the attempt to demonstrate the inter-relationship of various factors predisposing to SIDS. More precisely, we tested how one predisposing factor acts, provided that another predisposing factor is already present. Summing up these sub-divisions revealed no preponderance of SIDS characterized by (a) a low attendance for prenatal examinations by doctor and midwife, provided that the birth took place at home (Table 1), (b) home deliveries, provided that the mother was unmarried (cf. under Place of birth) or (c) young mothers, provided that the infant was premature (cf. under Age of parents). However, these unfavourable groups were small. On the other hand, there was a preponderance of SIDS cases characterized by (a) delivery at home, even provided that the birth order was higher

than one (Table 2), (b) low attendance for prenatal examinations, even provided that the birth order was higher than one (cf. under Prenatal care), (c) young mothers, even provided that they were unmarried (Table 4) and (d) high parity, even provided that the infants were premature (cf. under Parity). The finding that several predisposing factors are explainable by other factors supports the suspicion that a number of these perinatal factors are merely an indirect measure of unfavourable conditions and that continued research efforts might isolate a few factors of fundamental importance. The multivariate statistical methods which purport to be designed to unravel this type of complicated causal relations have regrettably given imperspicuous results (2, 12), possibly because of too small samples.

In pointing out risk groups with a view to prevention, it is important to bear in mind the lacking knowledge of the essential factors. Therefore, this type of prevention does not have much prospect of success at present.

In the present as well as in a previous paper (6), we have tried to elucidate the behaviour of some of the predisposing factors in the course of time. Among controls, we have demonstrated a decrease in deliveries at home, fewer infants born in wedlock (6) and a decrease in the person/room ratio (6). In other words, there has been a decrease in several unfavourable factors in the population without a demonstrable decrease in the incidence of SIDS during the same period (5). By following the case-control differences through time, it is seen that several factors show a distance in 1956-59 but a gradual approach up to 1968-71. These factors are place of birth (Table 3), birth order (Table 7), maternal age, maternal age corrected for parity (Table 11), father's occupation (6), mother's mental capacity (6) and the quality of infant care (6).

Thus, some of the factors emphasized here as well as in other papers as predisposing to SIDS have undergone a change in the course of time. This time trend contributes to the difficulty in defining risk groups.

Age at death

In all materials the distribution by age at death (1, 2, 7, 11, 15, 20, 22, 25) hrs shown the same tendency viz few deaths during the first month of life, a peak at two to four months followed by a rapid fall. Accordingly, by far the greater part of the deaths occur within the first six months, ranging in the available series from 65% to 100%.

Raring (19) pooled age at death data from seven materials. This gave a reasonably smooth curve, which he interpreted as being log normal. In his opinion, this supported the theory that SIDS was a separate distinct disease entity.

If SIDS is to be considered a disease entity it would be reasonable to compare it with the age at death curves for other disease groups. In our Fig. 2 it is very similar to the curve for deaths from respiratory tract infections. This indeed agrees with the findings that (a) respiratory symptoms are quite common during the days preceding death and that (b) there is an accumulation of deaths during the cold months (6). This is supported by an official report from England (16). Richards & McIntosh (20) compared the age at death for SIDS cases with those for children dying of gastroenteritis ($n=28$) and pneumonia ($n=36$) without being able to find any striking resemblance. The SIDS curve differs widely from that for other infant deaths considered together (Fig. 2) as is indeed well known (15).

An author from New Zealand (24) like us has reported that premature SIDS cases die later than mature ones, but the difference is not significant. In two publications (4, 10) it is merely mentioned without giving any figures that the age at death of prematures and matures does not differ significantly. These reflections are of importance in assessing the theory that SIDS occurs during a vulnerable period of physiological development corresponding to the characteristic narrow age distribution at death (11, 12) because if premature cases die later than mature cases this phenomenon may be interpreted to reflect that

prematures take longer to reach the vulnerable period.

Circumstances at death and time when found dead

That 13% died while sharing a bed with an adult supports the idea of instantaneous death without any death struggle (4). It would be of interest to compare the position when found with these and other infants, normal sleeping position and the position of other dead infants. This has been done, but the results have not been consistent (2, 4, 12).

Other materials (2, 4, 12, 23) support our finding that most of the infants are found dead in the morning or less often, in the afternoon. These hours correspond to the infants dying in their sleep during the night or during the midday nap. The sex difference in this respect like the other sex differences is not explicable.

CONCLUSIONS

In this and in the preceding paper (6) we have confirmed that SIDS families are under privileged as regards several social factors and perinatal factors of social nature. We have also pointed out that some of the factors can explain each other and we have emphasized the tendency for certain unfavourable factors not to accumulate any longer in SIDS cases and families. We have quoted the theory that a physiologically vulnerable period (11, 12) underlies SIDS which is suggested by the age curve and that various (unknown?) external factors need be only small to cause the infant's death if they occur during the vulnerable period. In view of this theory one might think that protection against or improvement of external factors would—at least to some extent—reduce the incidence of SIDS. One external factor might be for instance a trivial infection which again might be elicited by various influences of social nature—including older siblings as possible vectors.

But how is the constant incidence of SIDS

(5) to be reconciled with the finding that some predisposing factors are decreasing and that the case-control difference with respect to some factors has diminished? Have other predisposing factors become more prevalent during the same period thus maintaining *status quo*? Have new factors taken over? Or is it possible that the named factors used to be related to the actual cause(s) but no longer are—owing to the change in social structure? Not knowing the answers we can probably not alter the incidence of SIDS by improving social and perinatal conditions or by supervising special risk groups. Further information is needed and it might be of interest to ascertain how other predisposing and also apparently harmless factors alter in time as compared with the incidence of SIDS.

ACKNOWLEDGEMENTS

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REFERENCES

- Beal S. Sudden infant death syndrome. *Med J Aust* 2 1 3 1977.
- Beckwith J B. The sudden infant death syndrome. *Curr Probl Pediatr* 3 3 1973.
- Beckwith J B. In A B Bergman, J B Beckwith & C D Ray (eds). *Sudden infant death syndrome*. University of Washington Press, Seattle and London 1970 p 17.
- Bergman A B, Ray C G, Poterroy M A, Wahl P W & Beckwith J B. Studies of the sudden infant death syndrome in King County, Washington III. *Epidemiology Pediatrics* 49 860 1977.
- Biering Sørensen F, Jørgensen T & Hilden J. Sudden infant death in Copenhagen 1956-71. I. Infant feeding. *Acta Paediatr Scand* 67 179 1978.
- Biering Sørensen F, Jørgensen T & Hilden J. Sudden infant death in Copenhagen 1956-71. II. Social factors and morbidity. *Acta Paediatr Scand* 68 1 1979.
- Fedrick J. Sudden unexpected death in infants in the Oxford Record Linkage Area. An analysis with respect to time and place. *Br J Prev Soc Med* 27 217 1973.
- Fedrick J. Sudden unexpected death in infants in the Oxford Record Linkage Area. The mother. *Br J Prev Soc Med* 28 93 1974.
- Fedrick J. Sudden unexpected death in infants in the Oxford Record Linkage Area. Details of pregnancy, delivery and abnormality in the infant. *Br J Prev Soc Med* 28 164 1974.
- Froggatt P. Epidemiologic aspects of the Northern Ireland study. In Bergman A B, Beckwith J B & Ray C D (eds). *Sudden infant death syndrome*. University of Washington Press, Seattle and London 1970 p 37.
- Froggatt P, Lynas M A & MacKenzie M. Epidemiology of sudden unexpected death in infants (cot death) in Northern Ireland. *Br J Prev Soc Med* 25 119 1971.
- Froggatt P, Lynas M A & Marshall T W. Sudden unexpected death in infants (cot death). Report of a collaborative study in Northern Ireland. *Ulster Med J* 40 116 1971.
- Jørgensen T, Biering Sørensen F & Hilden J. Sudden infant death in Copenhagen 1956-71. IV. Infant development. To be published.
- Kraus A S, Steele R, Thompson M G & deGrosbois P. Further epidemiologic observations on sudden unexpected death in infancy in Ontario. *Can J Publ Health* 62 210 1971.
- Kraus J F & Borhani N O. Post neonatal sudden unexplained death in California. A cohort study. *Am J Epidemiol* 95 497 1972.
- Ministry of Health. Enquiry into sudden death in infancy. *Reports on Public Health and Medical Subjects No 113*. Her Majesty's Stationery Office, London 1965.
- Naeve R L, Ladis W & Drage J E. Sudden infant death syndrome. A prospective study. *Am J Dis Child* 130 1707 1976.
- Protestos C B, Carpenter R G, McWeeny P M & Emery J L. Obstetric and perinatal histories of children who died unexpectedly (cot death). *Arch Dis Child* 48 835 1973.
- Rating H H. SIDS—A note on age distribution of the syndrome. In R R Robinson (ed). *SIDS 1974 proceedings of the Francis E Camps international symposium on sudden and unexpected deaths in infancy*. The Canadian Foundation for the study of infant deaths, Toronto 1974 p 151.
- Richards I D G & McIntosh H T. Confidential inquiry into 766 consecutive infant deaths. *Arch Dis Child* 47 697 1972.
- Spiers P S. Previous fetal loss and risk of sudden infant death syndrome in subsequent offspring. *Am J Epidemiol* 103 355 1976.
- Steele W & Langworth J T. The relationship of antenatal and postnatal factors to sudden unexpected death in infancy. *Canad Med Assoc J* 94 1165 1966.
- Steele R, Kraus A S & Langworth J T. Sudden unexpected death in infancy in Ontario. Part I. Methodology and findings related to the host. *Can J Publ Health* 58 359 1967.

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But how is the constant incidence of SIDS

IgG SUBCLASS LEVELS IN INFANCY AND CHILDHOOD

VIVI ANNE OXELIUS

From the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Oxelius V (Department of Paediatrics University Hospital Lund Sweden) IgG subclass levels in infancy and childhood *Acta Paediatr Scand* 68 23 1979.—The concentrations of IgG1 IgG2 IgG3 and IgG4 were determined by electroimmunoassay in 10 pairs of maternal and cord sera and in sera of 162 healthy children aged 6 weeks to 15 years. Specific rabbit antisera against the IgG subclasses were used. The content of the normal serum pool WHO 67/97 was used as reference. The mean value, standard deviation and normal range of each IgG subclass were calculated for each age group and compared with the adult values. All IgG subclasses were present in cord serum except for IgG4 in those cases where also the maternal serum lacked demonstrable IgG4. The IgG subclasses followed the pattern of total IgG with a fall during the first 3-6 months and a subsequent gradual rise with age. The IgG1 and IgG3 levels rose faster with age than IgG2 and IgG4. Adult levels were not reached before puberty. No IgG4 was detectable in 12-21% of the children above 7 years of age.

KEY WORDS Serum IgG subclasses childhood

Quantification of immunoglobulins in serum is one of the parameters used to assess the immunological competence of an individual. The normal range and variation of concentrations of the different immunoglobulin classes in serum is wide in children and in adults. It is further known that the concentrations are lower in childhood than in adult age and that the age at which the adult level is reached varies from class to class (1, 2, 3, 4, 19, 20, 23).

Human IgG consists of four subclasses based on antigenic differences in their heavy polypeptide chains (22). The proportions of the IgG subclasses in a normal serum pool are IgG1 IgG2 IgG3 IgG4 60.9% 29.6% 5.3% 4.2% (11, 18). Imbalance of IgG subclasses and deficiency of IgG subclasses have been reported in children with recurrent infections (14, 17, 21, 25). Only a few studies are available on the levels of the IgG subclasses in healthy children (5, 6, 9, 10). The purpose of this investigation was to assess the variation of IgG subclass levels in normal Swedish children from birth to 15 years of age.

MATERIALS AND METHOD

Umbilical cord blood from 10 fullterm newborns and venous blood from their mothers were examined. An other 10 cord samples from fullterm newborns and venous blood samples from 162 children aged 6 weeks to 15 years were also examined. The main part of the older children were inpatients admitted to the Children's Department of Surgery because of hernia inguinalis, hydrocele testis, hernia femoralis and other surgical non-immunological diseases. One criterion for admission was that the children should be free from infection. Only patients with an E.S.N. below 10 mm/hr were included in the present material. Venous blood samples were also obtained from healthy infants from the Baby Welfare Center. Age distribution within the groups is shown in Table 1. Venous blood from 20 normal adults was included in the material. The serum samples obtained were kept at -20°C until analysed.

A normal serum pool of 400 blood donors with an IgG content of 17.8 IU/ml was used. The reference was the WHO pool 67/97 with an IgG content of 96 IU/ml. In the reference serum pool 67/97 the IgG subclasses have been determined to 5.1 g/l for IgG1, 2.5 g/l for IgG2, 0.55 g/l for IgG3 and 0.35 g/l for IgG4 (18). The values in g/l given in this article were according to the values reported for the reference WHO pool 67/97. The IgG subclasses of our normal serum pool (100%) were calculated to IgG1 6.8 g/l, IgG2 3.3 g/l, IgG3 0.73 g/l and IgG4 0.47 g/l.

Specific IgG subclass antisera prepared in rabbits as described earlier (13) were used. The specificity of antisera were tested against purified myeloma proteins of all

- 24 Tonkin M Epidemiology of SIDS in Auckland New Zealand In R R Robinson (ed) *SIDS 1974 proceedings of the Francis E Camps international symposium on sudden and unexpected deaths in infancy* The Canadian Foundation for the study of infant deaths Toronto 1974 p 169
- 25 Valdes Dapena M A Sudden and unexpected death in infancy A review of the world literature 1954-66 *Pediatrics* 39 123 1967
- 26 Valdes Dapena M McGovern J A Birle L J & Auerbach V H Changes in annual incidence

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A normal serum pool of 400 blood donors with an IgG content of 178 IU/ml was used. The reference was the WHO pool 67/97 with an IgG content of 96 IU/ml. In the reference serum pool 67/97 the IgG subclasses have been determined to 5.1 g/l for IgG1 2.5 g/l for IgG2 0.55 g/l for IgG3 and 0.35 g/l for IgG4 (18). The values in g/l given in this article were according to the values reported for the reference WHO pool 67/97. The IgG subclasses of our normal serum pool (100%) were calculated to IgG1 6.8 g/l IgG2 3.33 g/l IgG3 0.73 g/l and IgG4 0.47 g/l.

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- 26 Valdes Dapena M., McGovern J. A., Birle L. J. & Auerbach V. H. Changes in annual incidence

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Age	S.D.	Range
0-1	0.18	<0.01-0.47
1-4	0.14	<0.01-0.33
5-9	0.04	<0.01-0.14
10-14	0	<0.01
15-19	0.19	<0.01-0.65
20-24	0.37	<0.01-1.16
25-29	0.36	<0.01-1.1
30-34	0.25	<0.01-0.84
35-39	0.25	<0.01-1.21
40-44	0.33	<0.01-1.68
45-49	0.54	<0.01-1.68
50-54	0.77	<0.01-0.83
55-59	0.63	<0.01-0.91

strable IgG4. The mean value for IgG4 as well as for the other IgG subclasses then slowly increased with age. The concentration of IgG1 and IgG3 reached 50% of average adult concentration before 3 years of age; those of IgG2 and IgG4 after this age (non detectable IgG4 was regarded as 0.005 g/l). The concentration of IgG4 varied most and that of IgG1 least. Normal adult values were not reached by the oldest age group tested at 13-15 years of age. IgG4 was still non detectable in 12-21% of the children above 7 years of age.

DISCUSSION

The electroimmunoassay has proved a sensitive and accurate method for evaluation of the content of IgG subclasses in human serum (15). Good agreement was found between our results and those on record (6-9). The normal ranges of variation of IgG subclasses at different ages were estimated in order to facilitate the diagnosis of suspected IgG subclass deficiency conditions in children.

All the four IgG subclasses were found in cord sera. However, when the mother has no serum IgG4, the newborn constantly had no IgG4 in cord serum. This was evidence of the passive transfer of IgG4 across the placenta.

The IgG subclasses were not found in the

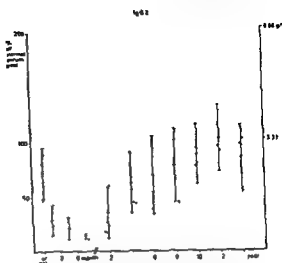


Fig. 3 The concentration relative to that of a normal serum pool (IgG 1.8 g/l) and in g/l of IgG1 in each individual compared with mean value \pm S.D. in age groups.

same proportions in cord serum as in the maternal serum. This means that the difference in concentrations between cord serum and maternal serum could not be explained entirely by plasma volume differences at birth. In every case the IgG1 cord serum level was higher than the level in the maternal serum. This is consistent with an active placental transport of IgG1 as is already described for total IgG (8). The IgG2, IgG3 and IgG4 cord serum levels were either higher or lower than the corresponding maternal serum levels. These findings indicate differences in transport mechanisms between the IgG subclasses. A selective transfer of different Gm factors across the placenta was shown already by Hunger & Thierbach (7). The child's own IgG synthesis starts during intra uterine life (13) but judging from studies of Gm factors, the amount produced is negligible compared with the amount originating from the mother. Concerning the metabolism of the four IgG subclasses it is roughly equal except for IgG3 (12) which has a biological half life of only one third of that of any of the other IgG subclasses.

All the serum IgG subclasses increase slowly

Table 1 IgG subclass levels in g/l in 182 healthy children compared with those in adults

Age	No	IgG1			IgG2			IgG3		
		Mean	S D	Range	Mean	S D	Range	Mean	S D	Range
Cord sera	20	6.02	1.23	4.35-10.84	2.11	0.76	1.43-4.53	0.59	0.32	0.27-1.46
0-2 months	7	3.67	1.02	2.18-4.96	0.93	0.47	0.40-1.67	0.16	0.08	0.04-0.73
3-5 months	9	3.06	0.82	1.43-3.94	0.70	0.33	0.23-1.47	0.30	0.29	0.04-1.00
6-8 months	8	2.99	0.75	1.90-3.88	0.47	0.07	0.37-0.60	0.32	0.16	0.17-0.67
9 months-2 years	24	4.49	1.09	2.86-6.80	1.17	0.77	0.30-3.27	0.42	0.21	0.13-0.87
3-4 years	22	5.58	1.02	3.81-8.84	2.03	0.90	0.70-4.43	0.51	0.22	0.17-0.90
5-6 years	20	4.83	1.36	2.92-8.16	2.27	1.17	0.83-5.13	0.48	0.32	0.08-1.11
7-8 years	17	5.71	1.16	4.22-8.02	2.57	1.10	1.13-4.80	0.61	0.34	0.15-1.55
9-10 years	20	6.73	1.22	4.56-9.38	2.90	0.90	1.63-5.13	0.67	0.21	0.26-1.13
11-12 years	16	6.46	1.29	4.56-9.52	3.37	1.00	1.47-4.93	0.64	0.44	0.17-1.79
13-14 years	19	6.39	1.56	3.47-9.93	2.80	0.97	1.40-4.40	0.69	0.34	0.23-1.17
Adults	20	7.55	2.45	4.22-12.92	3.80	1.50	1.17-7.47	0.73	0.26	0.41-1.79

Amounts less than 0.01 g/l regarded as 0.005 g/l

IgG subclasses in Ouchterlony technique and immunoelectrophoresis (15)

For quantitation of IgG subclasses in sera the electroimmunoassay was used as described earlier (15). The sensitivity of the method is about 0.01 g/l for all IgG subclasses.

RESULTS

All IgG subclasses were present in the cord sera except for IgG4 in those cases where the mother had no demonstrable IgG4 (<0.01 g/l).

in the serum. The IgG1 serum levels were regularly higher in the newborns than in the mothers. The serum IgG2, IgG3 and IgG4 concentrations in the newborns were either higher or lower than those in the mothers (Fig. 1).

The IgG subclass values found for different age groups are given in Figs. 2-5 and in Table 1. The lowest values were noted in the age groups 0-2 to 6-8 months. In the latter age group none of the children had any demon-

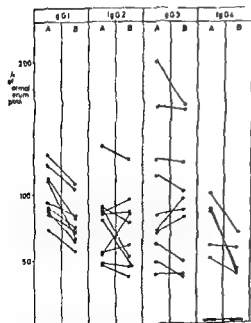


Fig. 1 The concentration of IgG subclasses in 10 cord (A) and maternal (B) sera.

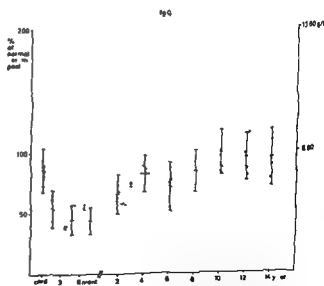


Fig. 2 The concentration relative to that of a normal serum pool (IgG 128 IU) and in g/l of IgG1 in each individual compared with mean value ± 1 SD in age groups.

REFERENCES

- 1 Allansmith M McClellan R H Butterworth M & Maloney I R The development of immunoglobulin levels in man *J Pediatr* 72 276 1968
- 2 Berg T & Johansson S G O Immunoglobulin levels during childhood with special regard to IgE *Acta Paediatr Scand* 58 513 1969
- 3 Collins-Williams C Tkachyk J J Toft H & Moscarello M Quantitative immunoglobulin levels (IgG, IgA and IgM) in children *Int Arch Allergy* 31 94 1967
- 4 Geny B Rodary C Griscelli P & Mozziconacci P Immunoglobulin D (IgD) in childhood I Maturation of serum IgD study of a population of healthy children *Biomedicine* 20 40 1974
- 5 Giessen M van der Freyee W Rossouw E & Loghem E van Qualitative and quantitative studies of IgG⁺ globulins in individual human sera with an antiserum capable of differentiating between Gm(n+) and Gm(n-) proteins *Clin Exp Immunol* 14 127 1973
- 6 Giessen M van der Rossouw E Algra van Veen T Loghem E van Zegers B J M & Sander P C Quantification of IgG subclasses in sera of normal adults and healthy children between 4 and 17 years of age *Clin Exp Immunol* 21 501 1975
- 7 Hunger H & Thuerbach V Gm(a) Bestimmungen bei Müttern und Neugeborenen *Blut* 9 385 1963
- 8 Kohler F F & Farr R S Elevation of cord over maternal IgG immunoglobulin Evidence for an active placental transport *Nature* 210 1070 1966
- 9 Morell A Skvarl F Hitzig W H & Barandun S IgG subclasses Development of the serum concentrations in normal infants and children *J Pediatr* 80 960 1971
- 10 Morell A Skvarl F Steinberg A G Loghem E van & Terry W H Correlations between the concentrations of the four subclasses of IgG and Gm allotypes in normal sera *J Immunol* 108 195 1971
- 11 Morell A Skvarl F & Barandun S *IgG subclasses der menschlichen Immunoglobuline* Karger Basel München Paris London New York and Sydney 1975
- 12 Morell A Terry W D & Waldmann T A Metabolic properties of IgG subclasses in man *J Clin Invest* 49 673 1970
- 13 Mårtensson L & Fudenberg H H Gm genes and γ G-globulin synthesis in the human fetus *J Immunol* 94 514 1965
- 14 Ovelius V A Chronic infections in a family with hereditary deficiency of IgG⁺ and IgG⁴ *Clin Exp Immunol* 17 19 1974
- 15 Ovelius V A Crossed immunoelectrophoresis and electromun assay of human IgG subclasses *Acta Path Microbiol Scand Sect C* 86 109 1978
- 16 Peltola H Kayhty H Sivenon A & Makela F H *Haemophilus influenzae* type III capsular polysaccharide vaccine in children A double blind field study of 100 000 vaccinees 3 months to 5 years of age in Finland *Pediatrics* 60 730 1977
- 17 Schur P H Borel H Gelfand E W Alper C & Rosen F Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections *N Engl J Med* 283 631 1970
- 18 Steinberg A G Morell A Skvarl F & Loghem E van The effect of Gm(?) on the concentration of IgG⁺ and IgG⁴ in normal human serum *J Immunol* 110 164 1973
- 19 Stuchm H & Fudenberg H H Serum levels of immunoglobulins in health and disease *Pediatrics* 37 715 1966
- 20 Stoop J W Zegers B J M Sander P C & Balieux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immunol* 4 101 1969
- 21 Terry W D Variations in the subclasses of IgG in R A Good & D Bergsma (eds) *Immunologic deficiency diseases in man Birth defects* The National Foundation 1968 p 357
- 22 Terry W D & Fahey J L Subclasses of human gamma-globulin based on differences on the heavy polypeptide chains *Science* 20 870 1964
- 23 Uffelman J A Engelhard W E & Jolliff C R Quantitation of immunoglobulins in normal children *Clin Chim Acta* 28 185 1970
- 24 Yount W J Dörner M M Kunkel H G & Kabat E A Studies on human antibodies VI Selective variations in subgroup composition and genetic markers *J Exp Med* 127 633 1968
- 25 Yount W J Hong S Sehgal M Good R A & Kunkel H G Imbalances of gammaglobulin subgroups and gene defects in patients with primary hypogammaglobulinemia *J Clin Invest* 49 1957 1970

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IgG3

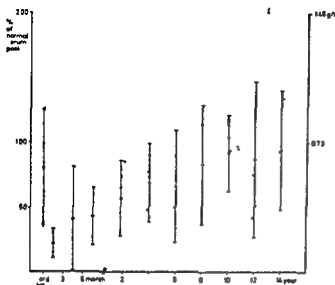


Fig. 4 The concentration relative to that of a normal serum pool (IgG 128 IU) and in g/l of IgG3 in each individual compared with mean value \pm SD in age groups

IgG4

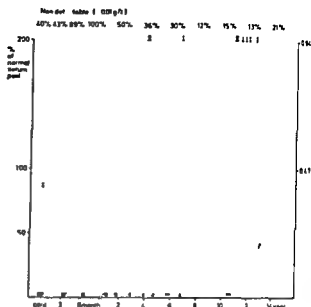


Fig. 5 The concentration relative to that of a normal serum pool (IgG 128 IU) and in g/l of IgG4 in each individual compared with mean value \pm SD in age groups. The frequency of individuals lacking IgG4 (IgG4 < 0.01 g/l) is given for each age group. Amounts less than 0.01 g/l regarded as 0.005 g/l

ly during childhood. Like others (9) we found that IgG1 and IgG3 increased faster with age than did IgG2 and IgG4. This means that the cell population responsible for each IgG subclass mature at different ages of the growing child. It is well known that specific antibodies belong to different IgG subclasses (24). The young child has not full immunological competence to make antibodies when stimulated for example by *Haemophilus influenzae* type b capsular polysaccharide vaccine (16). One of the reasons could be the delay in the synthesis of IgG2 and IgG4. We have earlier shown that antibodies to *Haemophilus influenzae* type b polysaccharide could not be found in patients with IgG2 and IgG4 deficiency (14).

The demonstrable IgG4 varied widely with in each age group from zero to very high values. Even in the higher age groups IgG4 was still undetectable in 12–21% IgG4 and IgD are quantitatively comparable. Both IgG4 and IgD are found in relatively low concentration in serum both are undetectable in about

25% of normal individuals and both have a wide normal range of variation.

IgG4 deficiency was shown to be very common in normal individuals. IgG1 is quantitatively the biggest of the IgG subclasses. IgG1 deficiency should therefore be suspected when a low IgG value is found. IgG2 or IgG3 deficiency could be found in patients with low or normal serum IgG. However it should be born in mind that IgG subclass deficiencies could be found also in patients with high IgG values. IgG subclass deficiencies should also be suspected when there is a restricted heterogeneity of immunoglobulins by electrophoresis of serum or when a deficiency of specific antibodies in serum can be proved. Because of continuously increasing serum levels in childhood it is advisable to repeat IgG subclass determinations after an interval of some months.

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REFERENCES

- 1 Allansmith M McClellan R H Butterworth M & Maloney J E The development of immunoglobulin levels in man *J Pediatr* 72 776 1968
- 2 Berg T & Johansson S O Immunoglobulin levels during childhood with special regard to IgE *Acta Paediatr Scand* 58 513 1969
- 3 Collins Williams C Tkachyk S J Toft H & Moscarello M Quantitative immunoglobulin levels (IgG IgA and IgM) in children *Int Arch Allergy* 31 94 1967
- 4 Geny B Rodary C Griscelli P & Mozziconacci P Immunoglobulin D (IgD) in childhood I Maturation of serum IgD study of a population of healthy children *Biomedicine* 20 40 1974
- 5 Giessen M van der Freywe W Rossouw E & Loghem E van Qualitative and quantitative studies of IgG globulins in individual human sera with an antiserum capable of differentiating between Gm(n+) and Gm(n-) proteins *Clin Exp Immunol* 14 177 1973
- 6 Giessen M van der Rossouw E Algra van Veen T Loghem E van Zegers H J M & Sander P C Quantification of IgG subclasses in sera of normal adults and healthy children between 4 and 17 years of age *Clin Exp Immunol* 21 501 1975
- 7 Hunger H & Thierbach V Gm(a) Bestimmungen bei Müttern und Neugeborenen *Blut* 9 385 1963
- 8 Kohler P F & Farr R S Elevation of cord over maternal IgG immunoglobulin Evidence for an active placental transport *Nature* 210 1070 1966
- 9 Morell A Skviri F Hitzig W H & Barandun S IgG subclasses Development of the serum concentrations in normal infants and children *J Pediatr* 80 960 1971
- 10 Morell A Skviri F Sternberg A G Loghem E van & Terry W H Correlations between the concentrations of the four subclasses of IgG and Gm allotypes in normal sera *J Immunol* 108 195 1972
- 11 Morell A Skviri F & Barandun S IgG subclasses der menschlichen Immunoglobuline S Karger Basel München Paris London New York and Sydney 1973
- 12 Morell A Terry W D & Waldmann T A Metabolic properties of IgG subclasses in man *J Clin Invest* 49 673 1970
- 13 Mårtensson L & Fudenberg H H Gm genes and YG-globulin synthesis in the human fetus *J Immunol* 94 514 1965
- 14 Orelus V A Chronic infections in a family with hereditary deficiency of IgG¹ and IgG4 *Clin Exp Immunol* 17 19 1974
- 15 Orelus V A Crossed immunoelectrophoresis and electroimmuno assay of human IgG subclasses *Acta Path Microbiol Scand Sect C* 86 109 1978
- 16 Peltola H Kayhty H Sivenon A & Makela P H Haemophilus influenzae type b capsular polysaccharide vaccine in children A double blind field study of 100 000 vaccinees 3 months to 5 years of age in Finland *Pediatrics* 60 730 1977
- 17 Schur P H Borel H Gelfand E W Alper C & Rosen F E Selective gamma-G globulin deficiencies in patients with recurrent pyogenic infections *Engl J Med* 283 631 1970
- 18 Sternberg A G Morell A Skviri F & Loghem E van The effect of Gm(23) on the concentration of IgG2 and IgG4 in normal human serum *J Immunol* 110 1647 1973
- 19 Stiehm E H & Fudenberg H H Serum levels of immunoglobulins in health and disease *Pediatrics* 37 715 1966
- 20 Stoop J W Zegers H J M Sander P C & Ballieux R E Serum immunoglobulin levels in healthy children and adults *Clin Exp Immunol* 4 101 1969
- 21 Terry W D Variations in the subclasses of IgG In R A Good & D Bergsma (eds) *Immunologic deficiency diseases in man Birth defects The National Foundation* 1968 p 357
- 22 Terry W D & Fahey J L Subclasses of human gamma-globulin based on differences on the heavy polypeptide chains *Science* 20 870 1964
- 23 Uffelman J A Engelhard W E & Jolliff C R Quantitation of immunoglobulins in normal children *Clin Chim Acta* 28 185 1970
- 24 Yount W J Dörner M M Kunkel H G & Kabat E A Studies on human antibodies VI Selective variations in subgroup composition and genetic markers *J Exp Med* 127 633 1968
- 25 Yount W J Hong M Seligman M Good R A & Kunkel H G Imbalances of gammaglobulin subgroups and gene defects in patients with primary hypogammaglobulinemia *J Clin Invest* 49 1957 1970

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BREAST MILK IRON—A DECLINING CONCENTRATION DURING THE COURSE OF LACTATION

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ABSTRACT Siimes M A, Vuori E and Kuitunen P (Children's Hospital and Department of Public Health Science, University of Helsinki, Helsinki, Finland). Breast milk iron—a declining concentration during the course of lactation. *Acta Paediatr Scand* 68: 29-31, 1979.—The present investigation is the first longitudinal study of the concentration of iron in breast milk and is based on 229 milk samples obtained from 27 mothers during their period of lactation up to 9 months. The samples were collected at the beginning and at the end of each feed during a 24-h period to reflect as accurately as possible the actual concentration of iron. The median value declined during the course of lactation from 0.6 to 0.3 mg/l with a large range of values. The results indicate that the concentration is lower than is generally stated or is unusually low in Finnish mothers. As a consequence some infants may require iron supplementation during prolonged breast feeding although in general the high bioavailability of breast milk iron prevents the development of iron deficiency.

KEY WORDS Human milk, lactation, iron

The available data on the concentration of iron in breast milk indicate that the mean values ranged from 0.6 to 1.3 mg/l in the 50's (2-4) and from 0.6 to 1.0 mg/l in the 60's (3-6) and from 0.2 to 0.8 mg/l in the 70's (1-9-11). The declining concentration may reflect a real phenomenon or it may be due to differences in methods of assay and sampling. At present the methods of determining iron by sensitive flame atomic absorption photometry (AAS) is generally considered valid. However the methods of obtaining the milk samples may influence the results since various iron concentrations for milk samples are reported in the literature and this difference in concentration is greater than that of some other minerals (11) since diurnal variation (11) and declining concentrations due to the stage of lactation play a role (6).

We feel there is a need for a longitudinal study in which milk samples are obtained in order to reflect as closely as possible the actual intake of iron and that such samples should be adequately analyzed. This question

has attained actuality since the bioavailability of iron in breast milk has been observed to be unusually high i.e. of the magnitude of 50% in infancy (14). The increasing knowledge of the range of the concentrations and the availability of iron in breast milk may serve as a basis for deciding whether iron supplementation is necessary in prolonged breast fed infants.

SUBJECTS AND METHODS

In the present study a group of 27 breast feeding mothers was followed up after a 7 week period of lactation. Milk aliquots of 8 ml were obtained at the beginning and at the end of each feed during a 24 h period (17). This method of collection allowed the mothers to continue the breast feeding of their infants and eliminated the diurnal variation and partly the potential variation during a single feeding. A total of 279 24 h samples from about 50-110 ml each were obtained during the nine months of lactation from 27 mothers. Initially the samples were obtained at intervals of 1-2 weeks and after lactation of 7 months every 3rd-4th week. The number of breast feeding mothers gradually declined to 13 at 6 months of lactation. By 9 months there were only two mothers left in the study. Over 60% of the mothers received iron supplementation daily after their delivery for the period of the present study. In ad-

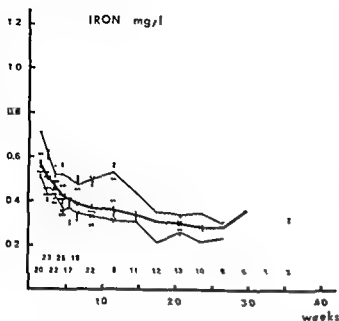


Fig 1 Iron concentration in 229 breast milk samples obtained from 27 mothers during a 9 month period of lactation. The total range and the 25th, 50th and 75th per centiles are shown of samples at 2-7 weeks of lactation and three weekly thereafter. The mean values were 0.01-0.07 mg/l higher than the medians. Numerals indicate the number of samples in each interval of time.

dition approximately half of the remaining mothers received iron medication irregularly.

The iron concentration in the milk samples frozen for a period of 2 to 6 weeks before analysis was determined from dry ash solution (17) by the flame AAS method (Perkin Elmer model 300). Bovine liver, a reference material 1577 from the National Bureau of Standards, Washington, D.C. was used as a control. Four determinations resulted in values ranging from 294 to 297 $\mu\text{g/g}$ dry weight and was slightly above the certified value of $270 \pm 20 \mu\text{g/g}$ dry weight. A pooled milk sample was analyzed 11 times and resulted in a mean of 0.39 mg/l between series with a S.D. value of 0.01 mg/l.

RESULTS

The median concentration of iron was 0.56 mg/l in the breast milk samples at two weeks of lactation (Fig. 1). Thereafter the value gradually declined and reached a plateau at a value of approximately 0.3 mg/l after 5 months of lactation. The mean values were generally higher due to the skewed distribution of the values. There was a marked individual variation from the lowest single value of 0.11 mg/l to the highest one of 1.14 mg/l (Fig. 1). The variation in concentration was greater before 3

months of lactation and varied especially within the high values for this period as indicated by the relatively narrow 50% range in Fig. 1.

There was also a tendency to maintain the individual concentrations as indicated by the finding that 13 of the 23 mothers breast feeding in the third month the concentration of iron remained in the same initial quartile and additionally 7 mothers remained within the range of \pm one quartile of the initial value.

DISCUSSION

The present investigation is the first longitudinal study of the concentration of iron in breast milk. The results indicate that the concentration in one half of the cases is initially within the range of 0.52 and 0.69 mg/l and later during the course of prolonged lactation averages approximately 0.3 mg/l. The decrease in the concentration is in accordance with a similar pattern in the concentration of lactoferrin (7). On the other hand, mean values rather than medians have been used consistently in all previous studies (1-6, 9-11) which has resulted in overestimating the concentration of iron due to the skewed distribution of the values.

Stored iron (16) and the concentration of hemoglobin (13) in infants reach the low and the lowest level respectively by the age of 6 months at the time when the concentration of iron in breast milk has also reached a low plateau as indicated in the present study. Following from the three aforementioned observations one may raise the question how long a period of time an infant can maintain an optimal iron nutrition while he is exclusively or mainly fed on breast milk. On the other hand, several recent reports indicate that the bioavailability of breast milk iron is unusually high (8, 13, 15) especially in exclusively breast fed infants (15). The available data also suggest that breast feeding up to a period of 6 months is not associated with a risk of iron de-

iciency (12) However such a risk might exist in some individual infants whose mother's milk iron concentration remains at a lower range of values in prolonged breast feeding or after an early introduction of solid foods

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REFERENCES

- 1 Anon The composition of mature human milk *Report on Health and Social Subjects 12* Her Majesty's Stationery Office London 1977
- 2 Belavady B & Gopalan C Chemical composition of human milk in poor Indian women *Indian Jour Med Res* 47 734 1959
- 3 Cavell P A & Widdowson E M Intakes and excretions of iron copper and zinc in the neonatal period *Arch Dis Child* 39 496 1964
- 4 Feuillen Y M & Plumier M Iron metabolism in infants 1 The intake of iron in breast feeding and artificial feeding (milk and milk foods) *Acta Paediatr Scand* 41 138 1957
- 5 Kasanen A & Ekman E M The iron content of breast milk and its variations *Duodecim* 71 855 1955
- 6 Kleinbaum H Über den Kupfergehalt der Nahrungsmittel des Kindes *Z Kinderheilkd* 86 655 196
- 7 Lonnerdal M Forsum E Gebre Medhin M & Hambræus L Breast milk composition in Ethiopian and Swedish mothers II Lactose nitrogen and protein content *Am J Clin Nutr* 29 1134 1976
- 8 McMullan J A Landaw M A & Oski F A Iron sufficiency in breast fed infants and the availability of iron from human milk *Pediatrics* 58 686 1976
- 9 Murthy G K & Rhea U S Cadmium copper iron lead manganese and zinc in evaporated milk infant products and human milk *J Dairy Sci* 54 1001 1971
- 10 Nassi L Poggini G Vecchi C & Galvan P Notes on the zinc copper and iron content of human colostrum and milk *Minerva Pediatr* 26 83 1974
- 11 Picciano M F & Guthrie H A Copper iron and zinc contents of mature human milk *Am J Clin Nutr* 29 24 1976
- 12 Saarinen U M Need for iron supplementation in infants on prolonged breast feeding *J Pediatr* 93 177 1978
- 13 Saarinen U M & Simes M A Developmental changes in red blood cell counts and indices of infant after exclusion of iron deficiency both by laboratory criteria and by continuous iron supplementation *J Pediatr* 97 41 1978
- 14 Saarinen U M Simes M A & Dallman P R Iron absorption in infants High bioavailability of iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin *J Pediatr* 91 36 1977
- 15 Saarinen U M & Simes M A Iron absorption from breast milk cow's milk and iron supplemented formula An opportunistic use of changes in total body iron determined by hemoglobin ferritin and body weight in 137 infants *Pediatr Res* (in press)
- 16 Simes M A Addiego J E & Dallman P R Ferritin in serum Diagnosis of iron deficiency and iron overload in infants and children *Blood* 43 481 1974
- 17 Vuori E & Kuitunen P The concentrations of copper and zinc in human milk—a longitudinal study *Acta Paediatr Scand* 68 33 1979

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THE CONCENTRATIONS OF COPPER AND ZINC IN
HUMAN MILK*A Longitudinal Study*

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ABSTRACT Vuori E and Kuitunen P (Department of Public Health Science and Children's Hospital University of Helsinki Helsinki Finland) The concentrations of copper and zinc in human milk—a longitudinal study *Acta Paediatr Scand* 68 33 1979.—Twenty-seven healthy Finnish mothers were followed during the course of their entire lactation period. A total of 229 individual milk samples collected in the beginning and at the end of each feed during a 24-h period were obtained from the 2nd week to the 9th month of lactation. The copper and zinc concentrations were determined by atomic absorption spectrophotometry. The concentrations of the trace elements investigated were dependent on the stage of lactation. The median copper and zinc concentrations decreased during the course of lactation from about 0.60 mg/l and 4.0 mg/l to 0.25 mg/l and 0.5 mg/l respectively. The importance of considering the stage of lactation in the evaluation of the trace-element nutrition value of breast milk should be emphasized. The calculated means of the concentrations of these trace-elements in mature human milk presented in the literature seem to overestimate the actual levels in prolonged lactation.

KEY WORDS Human milk lactation copper zinc

Earlier studies on the trace element content of human milk have shown considerable variations both from mother to mother and in the case of copper and zinc also as regards the stage of lactation (17). According to available data the copper levels have ranged from 0.15 to 1.34 mg/l (1-3, 5, 7, 11-15) and the zinc content from 0.65 to 5.3 mg/l (1, 3-5, 10, 13-15) on the average.

It has not been clarified to what extent these variable data really reflect individual variation and to what extent the variation may be due to the different stages of lactation or perhaps be due to the different methods of sampling. The problem is best solved by means of a longitudinal study and therefore we have followed up 27 mothers during their entire lactation period and have determined the concentrations of copper and zinc in human milk.

According to the current recommendations for infant feeding breast milk is preferred and during the first four to six months of life it

should fulfill the nutritional needs of the infant (8). The trace element content of breast milk when adequately sampled and analyzed ought to serve as a guide in the assessment of the nutritional needs of trace elements for the infant.

MATERIAL

Mothers

Forty-three primipara were asked if they would be interested in taking part in the study. Thirty-seven interested mothers were recontacted after discharge. A group of thirty mothers was obtained during April and May 1976. Two of the mothers were unable to supply milk samples and one other mother used a breast pump for collection and thus three mothers were excluded from the follow up. All mothers were healthy, well nourished and lived in the greater Helsinki area. The mean age of the mothers was 28 years with a range of 20 to 35 years.

Milk samples

A special bottle was designed for sampling the milk: a wide-mouthed 175 ml polyethylene bottle having a scale on the side, each division representing a volume of 8

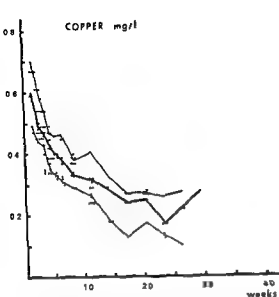


Fig 1 The average copper concentration of human milk during lactation. The figure gives the total range, median values and the 25th and the 75th percentiles after the 2nd week weekly and after the 7th week three weekly. Total number of samples is 9.

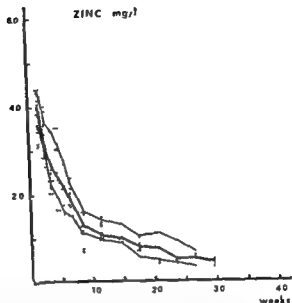


Fig 2 The average zinc concentration of human milk during lactation. The figure gives the total range, median values and the 25th and the 75th percentiles after the 2nd week weekly and after the 7th week three weekly. Total number of samples is 9.

0.10–0.35 mg/l lower than the means of copper and zinc concentrations respectively.

In the beginning of the study the median concentrations of copper and zinc were 0.60 mg/l and 4.0 mg/l. The trace-element concentrations investigated showed to be dependent on the stage of lactation (Figs 1–2). After 20 weeks of lactation the medians of copper and zinc values were about 0.25 mg/l for copper and 0.5 mg/l for zinc. The total ranges of the copper values were 0.03–0.75 mg/l and of zinc values 0.18–6.0 mg/l.

Table 4 allows a comparison of the copper and zinc concentrations in human milk presented together with some earlier data.

DISCUSSION

On the basis of this longitudinal study it can be stated that the earlier data on the average trace element content of human milk overestimate the actual levels in prolonged lactation. Mature human milk is said to contain 0.4 mg/l copper and 3–5 mg/l zinc on the aver-

age (6). However, 50% of our mothers had a copper content in their milk under the given value after 7 weeks of lactation and all of them after 14 weeks, and in the case of zinc after 4 and 9 weeks respectively. This obliquity is perhaps caused by the fact that most earlier studies have been made in the beginning of lactation (Table 4) and perhaps the previous reports on the effect of the stage of lactation on the trace element content of human milk have not been sufficiently convincing.

When studying human milk the first and perhaps the most difficult stage is to get representative samples. In previous studies all authors have collected their samples avoiding contamination, but only a few give information on how the samples were actually taken, e.g. before nursing the baby (15). Hytten has put forward very rigid requirements which a sample of milk has to fulfill: a pooled sample representing the entire milk production of a period covering 24 hours (9). However, in studies conducted outside the

Table 1 *The median copper and zinc concentrations of human milk and the number of samples from the 2nd to the 37th week of lactation*

Stage of lactation week	No of samples	Cu mg/l	Zn mg/l
2	20	0.60	4.0
3	23	0.49	3.0
4	22	0.47	2.5
5	25	0.42	2.4
6	17	0.40	2.1
7	18	0.38	1.9
8-10	22	0.33	1.3
11-13	18	0.32	1.1
14-16	11	0.28	0.95
17-19	12	0.24	0.78
20-22	13	0.25	0.75
23-25	10	0.17	0.49
26-28	9	0.22	0.52
29-31	8	0.27	0.44
32-34	1	0.24	0.42
35-37	2	0.25	0.49

ml. The mothers were advised to wash their breasts with plain water and to dry them afterwards. Before and after each feed the mothers took a sample (one interval on the scale) by manual milking directly into the sampling bottle. This was repeated every time the mother fed her baby over a period of 24 hours. Thus the final sample of milk obtained over a 24 h period consisted of foremilk and hindmilk in equal proportions. This method of collecting human milk has been used earlier e.g. by Tarján *et al.* (16).

During the first three months of lactation the mothers nursed their infants 5-7 times and later on 3-4 times during each 24 h period. Instructions were given to keep the bottle refrigerated during the collection of the samples. On the following day the bottle was taken to the laboratory for deep freezing. At first the mothers supplied milk samples at intervals of 1-2 weeks and after two months at intervals of 3-4 weeks. The mean number of samples supplied by the mothers was 8.6 with a range of 3-14. Thus the group of 27 mothers supplied 229 milk samples from the 2nd to the 37th week of lactation (Table 1). Samples from the last 6 weeks were excluded from the statistical analysis because the number of mothers and the number of milk samples supplied was small.

This method of collecting milk samples was found to be convenient and it did not disturb lactation.

METHODS

Milk samples which had been stored frozen from 2 to 6 weeks were warmed to body temperature in a water bath and mixed by gentle shaking. Ten milliliters of milk were measured into covered 50 ml silica crucibles. The samples were dried at 105 °C overnight. They were then

Table 2 *Accuracy of the method*

NBS Bovine Liver Standard Reference Material 1577
determinations µg/g dry weight

	Mean	Range	Certified value
Cu	200	199-200	193 ± 10
Zn	140	138-141	130 ± 10

carbonized in a muffle furnace at 250 °C for 4-5 hours and subsequently ashed at 450 °C for about 48 hours. In the middle of the ashing procedure the crucibles were allowed to cool and 0.1 ml of conc. HNO₃ (Suprapur[®] Merck) was added after which the temperature was again raised gradually to 450 °C. The ash was first dissolved with 0.5 ml of 6 M HCl (Suprapur[®] Merck) and then with 1 ml of 2 M HCl with gentle warming. The crucibles and covers were rinsed several times with small volumes of deionized water and finally diluted to 5 ml with deionized water.

The copper concentration in the milk sample was then determined by the flame Atomic Absorption Spectrophotometric (AAS) method (Perkin Elmer model 100) directly from the acid ash solution. For the determination of zinc content a further dilution (1+9) with deionized water was needed.

The accuracy of the method was tested by analyzing Bovine Liver Standard Reference Material No 1577 supplied by the National Bureau of Standards, Washington, D.C. (Table 2) and the precision of the method was established by repeated analysis of pooled human milk (Table 3).

RESULTS

Instead of using means and standard deviations as previously given by earlier authors we have expressed our results by giving the total ranges, the 25th and 75th percentiles and median values because the distribution of the trace element concentrations was found to be skewed. However the differences between the median and mean values were not great, the medians being 0.00-0.03 mg/l higher and

Table 3 *Precision of the method*

Between series calculated from 11 samples of pooled human milk mg/l

	Mean	S.D.
Cu	0.41	0.02
Zn	2.5	0.1

75th percentiles Picciano & Guthrie also found the skewed distributions of copper and zinc in their material but in both cases towards lower values (15). In the light of the present longitudinal study the stage of lactation must be emphasized. They had taken samples from the 6th to 12th weeks of lactation and according to our results the median of the copper content diminished from about 0.4 to 0.3 mg/l during the given period (Table 1). Thus the opposite skewed distribution might be caused by the preponderance of mothers who had nursed for a longer period.

The copper and zinc concentrations of human milk presented here are compatible with that of most previous reports when the stage of lactation and the number of samples are taken into account (Table 4). The present results suggest that no single value can be given for the concentrations of trace elements in human milk without relating it to the stage of lactation. As regards zinc and copper no mature milk seems to exist before the 5th–6th month of lactation. Earlier calculated means of the concentrations of trace elements of mature human milk as presented in the literature seem to overestimate the actual levels. Thus these values do not accurately reflect the supply of copper and zinc derived from human milk during prolonged breast feeding but the actual levels of trace elements as related to each stage of lactation should serve as a basis for determining the quantity of trace elements supplied to the infant.

ACKNOWLEDGEMENT

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REFERENCES

- 1 Anon The composition of mature human milk. *Report on Health and Social Subjects 12*. Her Majesty's Stationery Office London 1977

- 2 Beck A II see Underwood (17) p 74
- 3 Belavady II & Gopalan C Chemical composition of human milk in poor Indian women *Indian Jour Med Res* 47 234 1959
- 4 Berfenstam R Studies on blood zinc. A clinical and experimental investigation into the zinc content of plasma and blood corpuscles with special reference to infancy *Acta Paediatr Scand* 41 105 1952
- 5 Cavell P A & Widdowson E M Intakes and excretions of iron copper and zinc in the neonatal period *Arch Dis Child* 39 496 1964
- 6 Fomon W J *Infant nutrition* W B Saunders Company Philadelphia 1974 2nd ed
- 7 Grebenikov E P Soroka V R & Sabadash E V Trace elements in human and animal milk *Fed Proc* 23 461 1964
- 8 Hambræus L Proprietary milk versus human breast milk in infant feeding. A critical appraisal from the nutritional point of view *Pediatr Clin North Am* 24 17 1977
- 9 Hytten F E Clinical and chemical studies in human lactation *Br Med J* 1 175 1954
- 10 Jensen R I Thomas L M Bergman K E Filer L J Jr & Fomon S J 1977 see Fomon (6) p 364–365
- 11 Kleinbaum H Über den Kupfergehalt der Nahrungsmittel des Kindes *Z Kinderheilkd* 86 655 1967
- 12 Munch Petersen P On the copper content in mother's milk before and after intravenous copper administration *Acta Paediatr Scand* 9 378 1950
- 13 Murthy G K & Rhea U S Cadmium copper iron lead manganese and zinc in evaporated milk infant products and human milk *J Dairy Sci* 54 1001 1971
- 14 Nassi L Poggini G Vecchi C & Galvan P Notes on the zinc copper and iron content of human colostrum and milk *Minerva Paediatr* 26 832 1974
- 15 Picciano M F & Guthrie H A Copper iron and zinc contents of mature human milk *Am J Clin Nutr* 29 247 1976
- 16 Tärjån M Kramer M Szoke S & Lindner K The effect of different factors on the composition of human milk and its variation I *Nutr Diet* 3 1 1963
- 17 Underwood E J *Trace elements in human and animal nutrition* Academic Press New York 1971 3rd ed

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Table 4 The average copper and zinc concentrations of human milk according to different authors

Cu (mg/l)	Zn (mg/l)	No of samples	Stage of lactation	Reference
0.48	-	115	1st week	Munch Petersen 1950 (12)
-	5.3-3.6	?	4th day	Berfenstam 1952 (4)
-	11.65	?	6th month	
0.56	3.54	9-11	2nd-6th month	Belavady & Gopalan 1959 (3)
0.67-0.89	-	?	1st week after	Beck 1960 (2)
0.15-0.17	-	?	several months	
0.25	-	30	6th-7th day	Grebennikov et al 1961 (7)
1.34	-	15	1st week	Kleinbaum 1962 (11)
0.26	-	52	5th-6th month	
0.62	4.08	10	5th-8th day	Cavell & Widdowson 1964 (5)
0.24	1.34	22	?	Murthy & Rhea 1971 (13)
-	3.7	17 ^a	2nd week	Jensen et al 1972 (10)
-	1.1	12 ^a	18th week	
0.36	5.07	55 ^a	5th day	Nassi et al 1974 (14)
0.29	3.24	?	15th day	
0.24	1.63	350	6th-12th week	Picciano & Guthrie 1976 (15)
0.39	2.95	96	4th-6th week	Anon 1977 (1)
0.60 ^c	4.0 ^c	229	2nd week-31st week	Present study
0.27	0.44 ^c			

The authors give ppm in ash

^a Number of mothers

Median value

hospital when the same mothers are to be followed up during the entire lactation period such a method is difficult to follow. In the present study we have had to be satisfied with the second best but practical method: the milk samples represent every feed during a period of 24 hours: foremilk and hindmilk in equal proportions. Furthermore the mothers took the samples by manual milking directly into the sampling bottles. So we have tried to minimize both contamination and the effect of known variations in the components of milk.

All the mothers used in this study were primarily healthy and well nourished. The subjects volunteered for the study before breast feeding had begun. The fact that the breast feeding of this group of mothers was successful is partly attributable to the continuous encouraging to carry on breast feeding and partly due to the 6 month maternity leave which is now granted to mothers in Finland.

Both the copper and zinc concentrations of

human milk are reported to diminish during lactation (17). The individual variations are said to be great (17). Recently Picciano & Guthrie demonstrated diurnal variations as well: the copper and zinc levels seem to be higher in the morning than later in the same day (15). No significant difference has been found between the copper content of foremilk and hindmilk (12).

The average levels of trace element concentrations of human milk are given here using medians instead of means. This is done because the distributions of values were found to be skewed. Calculated mean values overestimate the actual average zinc and underestimate the copper content. However the differences between the medians and the means are not great, being at highest 10-15% divergent from the median values. But if the variation of individual values is expressed by giving the standard deviation, it overestimates the real variation because of the skewed distribution. So we have expressed the variation by giving the total ranges: the 25th and

OSTEOMYELITIS OF THE PUBIS

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ABSTRACT Heldrich F J and Harris V C (Department of Pediatrics St Agnes Hospital Baltimore Maryland USA) Osteomyelitis of the pubis. Acta Paediatr Scand 67 39 1979.—Three cases of osteomyelitis of the pubis are presented and the clinical and laboratory features summarized. A review of the literature revealed 49 cases and emphasizes the rarity of this location for acute primary osteomyelitis.

KEY WORDS Osteomyelitis pubis childhood

Pelvic osteomyelitis has been documented as occurring in 2% to 11% of patients of all ages reported in eight large series (4, 12, 13, 15, 17, 18). The pubic bone is involved least commonly of all of the bones of the pelvis; we were able to find 49 cases, including both children and adults, of osteomyelitis involving the pubic bone reported from 1883–1978 (5, 6, 8, 9, 12, 13, 16, 17). This paper reports the findings in three patients with acute primary osteomyelitis of the pubis. The rarity of this entity as well as the clinical presentation prompted this report.

CASE REPORTS

Case 1 M C, a 6½ year-old white male, admitted with limping and pain in the left hip of ten days' duration prior to high fever with delirium one day prior to admission. Four years and two years previously the child had experienced pain in the left hip lasting several days. Radiographs were negative and no therapy was instituted. Four weeks prior to this admission the patient again had pain in the left hip. Physical examination and hip radiographs were negative. Three weeks prior to admission lesions of erythema nodosum appeared on the lower extremities. A throat culture was positive for group A beta hemolytic *Staphylococcus* and erythromycin 250 mg every six hours was given for ten days. No history of trauma. Examination revealed temperature 39.6°C, pulse 128 beats per minute, respiratory rate of 4 breaths per minute, blood pressure 100/64 mmHg. There was limitation of voluntary movement of the left hip with marked resistance to active internal rotation. No point tenderness on palpation of the hip joint or trochanter. Fullness was apparent in the left

femoral triangle. Special studies included: Peripheral white cell count $37000/\text{mm}^3$, 74% neutrophils, 17% lymphocytes, 6 monocytes, 2 eosinophils, 1 band. Erythrocyte sedimentation rate 41. Blood cultures times 2 negative. Radiographs revealed a radiolucent lesion in the inferior ramus of the left pubic bone. Fifteen cc of pus were aspirated anterior and medial to the hip, which grew *Staphylococcus aureus*. An abscess outside the hip joint extending from the pubic bone was drained surgically. Intravenous methicillin was given for four weeks followed by oral erythromycin for two weeks. Four months later the patient was clinically well with radiologic improvement of the lesion in the pubic bone.

Case 2 M Y, a 9 year-old female, was admitted with pain in the right hip of five days' duration and a high fever for one day. Two weeks prior to admission she had complained of pain in the right thigh. Physical examination was normal at that time. On admission temperature 40°C, pulse 92 beats per minute, respiratory rate 20 breaths per minute, blood pressure 114/70 mmHg. The patient limped on the right hip. When prone the hip was held in external rotation and abduction. Palpation and passive movements of the hip were not painful. Laboratory data revealed: Peripheral leucocyte count $17400/\text{mm}^3$ with 66% neutrophils, 13% lymphocytes, 8% monocytes, 13% bands. Erythrocyte sedimentation rate 39. Initial radiographs of the hip, pelvis, femur and lumbosacral region were negative. Blood cultures times 3 were negative. Synovial fluid aspirated from the hip was sterile. The child remained symptomatic and was started on intravenous methicillin. On the seventh hospital day repeat radiographs revealed a lytic lesion in the inferior ramus of the right pubis. Total therapy consisted of ten days of intravenous methicillin and six weeks of oral cloxacillin. Nine months later the patient was doing well and radiographs revealed marked improvement (Fig. 1).

Case 3 C J, a 7 year-old white female, was admitted with sore throat for three days, high fever for two days and pain in the right hip one day prior to admission.

day after admission respectively. Shielding of the gonads in males during the X ray procedure may interfere with visualization of the pubic bone. Radioisotopic delineation of osteomyelitis may be possible earlier than conventional radiographs but Case 3 demonstrated that accumulation of the radioisotope in the bladder urine may interfere with the visualization of the bones of the pelvis.

Experience with three cases of acute primary osteomyelitis of the pubis has enabled us to summarize their salient features of presentation (Table 1).

Failure to detect osteomyelitis as the cause of illness may lead to inadequate therapy and thus predispose to chronic infection or other complications. Management should be in accordance with accepted standards for osteomyelitis (2, 7, 10, 14). Recovery of an organism preferably from the site of involvement should determine the choice of antibiotics. Organisms recovered were penicillin resistant *Staphylococcus aureus* by tissue aspiration in Case 1 and a group A beta hemolytic *Streptococcus* by blood culture in Case 3. All patients responded to the antibiotics chosen and recovery was satisfactory in each instance.

CONCLUSION

Osteomyelitis of the pubis while uncommon should be included in the differential diagnosis for patients presenting with symptoms suggestive of hip pathology. Our observations from these three reported cases suggest the following findings to be helpful:

- 1 Severe pain elicited on active motion of the hip but not on passive motion.
- 2 Absence of radiologic confirmation of hip involvement (including soft tissue signs).
- 3 Normal synovial fluid on hip aspiration.
- 4 Tenderness over the pubic area.

REFERENCES

- 1 Aksby R L & Stabek L T. Pyogenic infections of the sacroiliac joint in children (Radioisotope bone

- scanning as a diagnostic tool) *Clin Orthop* 100: 96, 1974.
- 2 Chalkin V D. Management of severe forms of haematogenous osteomyelitis of the pelvic bone. *Orthop Traumatol Prole* 77: 29, 1966 (Russ.).
- 3 Chung S M K & Boron P. Acute osteomyelitis adjacent to the sacroiliac joint in children (Report of 2 cases). *J Bone Joint Surg* 55A: 630, 1973.
- 4 Dieh V Q, Nelson J D & Haktain K C. Osteomyelitis of infants and children. A review of 163 cases. *Am J Dis Child* 129: 1773, 1975.
- 5 Dinan J J. Two cases of primary osteomyelitis of the pubic bone. *Can Med Assoc J* 41: 436, 1939.
- 6 Edwards M S, Baker C J, Granberry W M & Barrett F F. Pelvic osteomyelitis in children. *Pediatrics* 61: 67, 1978.
- 7 Ferguson A H Jr. Osteomyelitis in children. *Clin Orthop* 96: 51, 1973.
- 8 Fourrier A, Pauls A, Ducoulombier H, Cousin J & Huguet J. Osteomyelitis of the pubic bone. *J Sci Med Lille* 83: 391, 1967 (Fr.).
- 9 Geist E S. Osteomyelitis of pubic bone. *JAMA* 77: 1919, 1921.
- 10 Kulowski J, Hicks E O & Steindler A. Operative treatment of suppurative lesions of the bony pelvis. *Am J Surg* 79: 405, 1950.
- 11 Leites R M, Afifi A & Sutherland J B. Technetium bone scanning as an aid in the diagnosis of atypical osteomyelitis in children. *Surg Gynecol Obstet* 140: 899, 1975.
- 12 McWhorter G. Osteomyelitis of the ischium and pubis. *Surg Gynecol Obstet* 49: 705, 1979.
- 13 Morgan A & Yates A K. The diagnosis of acute osteomyelitis of the pelvis. *Postgrad Med J* 42: 74, 1966.
- 14 Nelson J D. The bacterial etiology and antibacterial management of septic arthritis in infants and children. *Pediatrics* 50: 437, 1972.
- 15 Scott L. Osteomyelitis of the pelvis. *South Med J* 20: 104, 1927.
- 16 Waldvogel F A, Medoff G & Swartz M N. Osteomyelitis. A review of clinical features, therapeutic considerations and unusual aspects (Part I). *N Engl J Med* 282: 198, 1976.
- 17 Wilensky A O. Osteomyelitis of the pelvic girdle. *Arch Surg* 37: 371, 1938.
- 18 Young F. Acute osteomyelitis of the ilium. *Surg Gynecol Obstet* 58: 986, 1934.

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Fig. 1 Patient M Y (A) Lytic lesion in the inferior ramus of the right pubis (B) Follow up radiograph showing improvement 8 months later

Temperature was 40.4°C pulse 124 beats per minute respiratory rate 30 breaths per minute blood pressure 110/60 mmHg. The patient limped on the right hip which was held externally rotated, flexed and abducted with marked limitation of active movement. There was tenderness on palpation just lateral to the symphysis pubis on the right. Laboratory studies were: Peripheral leucocyte count 22,000/mm³ with 57% neutrophils, 3% lymphocytes, 1% monocytes, 39% bands. Erythrocyte sedimentation rate 44. Radiographs of the hip and pelvis were negative. Accumulation of dye in the urinary bladder on technetium scan obscured the pelvic girdle. Blood culture grew beta hemolytic *Streptococci* group A. Hip aspiration was negative. The child was started on intravenous methicillin and subsequently changed to penicillin G. On the 22nd day a radiograph revealed a lytic lesion in the inferior ramus of the right pubic bone. The patient received six weeks of intravenous antibiotics and four months later was doing well with demonstrated radiologic improvement.

Table 1 Summary of findings

	Case 1	Case 2	Case 3
<i>History</i>			
Fever	+	+	+
Hip pain	+	+	+
Pubic pain	-	-	-
Trauma	0	0	0
<i>Physical findings</i>			
Hip pain			
Active motion	+	+	+
Passive motion	-	-	+
Pubic tenderness	-	-	+
<i>Lab data</i>			
Leucocytosis	+	+	+
Elevated sed rate	+	+	+
X Rays			
Hip	-	-	-
Pubis	+	+	+
Cultures			
Blood	-	-	+
Pubis	+	0	0
Soft tissue	+	0	0
Hip joint	-	-	-

DISCUSSION

The initial diagnosis in all three cases on admission was septic arthritis of the hip. This condition requires early recognition and prompt surgical drainage to minimize permanent damage to the hip joint. Noteworthy in our cases is the fact that passive motion of the hip was reasonably well tolerated whereas active motion, particularly when weight bearing was not. In such instances osteomyelitis involving the metaphysis of the femur would be the most likely site of involvement. But as our cases indicate, the bones of the pelvic girdle may also be the site of pathology. In Case 3, our house staff, now alerted to this possibility, found tenderness over the pubis and made the provisional diagnosis.

Aids to the diagnosis of osteomyelitis include radiographs and radionuclide scans but these have limitations also (1, 11). Radiologic evidence of osteomyelitis is positive only 10 to 14 days or more after onset. In two of our cases, the radiographs did not become positive until the seventh and twenty second

day after admission respectively. Shielding of the gonads in males during the X ray procedure may interfere with visualization of the pubic bone. Radioisotopic delineation of osteomyelitis may be possible earlier than conventional radiographs but Case 3 demonstrated that accumulation of the radioisotope in the bladder urine may interfere with the visualization of the bones of the pelvis.

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REFERENCES

1. Aksby R L & Staheli L T. Pyogenic infections of the sacroiliac joint in children (Rad osotope bone

- scanning as a diagnostic tool) *Clin Orthop* 100: 96, 1974.
2. Chaklin V D. Management of severe forms of haematogenous osteomyelitis of the pelvic bone. *Orthop Traumatol Prote* 27: 29, 1966 (Russ.).
3. Chung S M K & Borns F. Acute osteomyelitis adjacent to the sacroiliac joint in children (Report of 7 cases). *J Bone Joint Surg* 55A: 610, 1973.
4. Dieh V Q, Nelson J D & Hatalin K C. Osteomyelitis of infants and children: A review of 163 cases. *Am J Dis Child* 129: 1773, 1975.
5. Dinan J J. Two cases of primary osteomyelitis of the pubic bone. *Can Med Assoc J* 41: 436, 1939.
6. Edwards M S, Baker C J, Granberry W M & Barrett F F. Pelvic osteomyelitis in children. *Pediatrics* 61: 67, 1978.
7. Ferguson A B Jr. Osteomyelitis in children. *Clin Orthop* 96: 51, 1973.
8. Fournier A, Pauli A, Ducoulombier H, Cousin J & Huguet J. Osteomyelitis of the pubic bone. *J Sci Med Lille* 85: 391, 1967 (Fr.).
9. Geist E S. Osteomyelitis of pubic bone. *JAMA* 77: 1939, 1971.
10. Kulowski J, Hicks E M & Steindler A. Operative treatment of suppurative lesions of the bony pelvis. *Am J Surg* 79: 405, 1950.
11. Lettis R M, Afifi A & Sutherland J B. Technetium bone scanning as an aid in the diagnosis of atypical osteomyelitis in children. *Surg Gynecol Obstet* 140: 899, 1975.
12. McWhorter G. Osteomyelitis of the ischium and pubis. *Surg Gynecol Obstet* 49: 705, 1979.
13. Morgan A & Yates A K. The diagnosis of acute osteomyelitis of the pelvis. *Postgrad Med J* 42: 74, 1966.
14. Nelson J D. The bacterial etiology and antibacterial management of septic arthritis in infants and children. *Pediatrics* 50: 437, 1972.
15. Scott L. Osteomyelitis of the pelvis. *South Med J* 20: 104, 1977.
16. Waldvogel F A, Medoff M & Swartz M N. Osteomyelitis: A review of clinical features, therapeutic considerations and unusual aspects (Part I). *Engl J Med* 282: 198, 1976.
17. Wilensky A O. Osteomyelitis of the pelvic girdle. *Arch Surg* 37: 371, 1918.
18. Young F. Acute osteomyelitis of the ilium. *Surg Gynecol Obstet* 58: 986, 1934.

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Fig 1 Patient M Y (A) Lytic lesion in the inferior ramus of the right pubis (B) Follow up radiograph showing improvement 9 months later

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Temperature was 40.4°C pulse 124 beats per minute respiratory rate 30 breaths per minute blood pressure 110/60 mmHg. The patient limped on the right hip which was held externally rotated flexed and abducted with marked limitation of active movement. There was tenderness on palpation just lateral to the symphysis pubis on the right. Laboratory studies were: Peripheral leucocyte count 22,000/mm³ with 57% neutrophils 3% lymphocytes 1% monocytes 39% bands. Erythrocyte sedimentation rate 44. Radiographs of the hip and pelvis were negative. Accumulation of dye in the urinary bladder on technetium scan obscured the pelvic girdle. Blood culture grew beta hemolytic *Streptococcus* group A. Hip aspiration was negative. The child was started on intravenous methicillin and subsequently changed to penicillin G. On the 22nd day a radiograph revealed a lytic lesion in the inferior ramus of the right pubic bone. The patient received six weeks of intravenous antibiotics and four months later was doing well with demonstrated radiologic improvement.

day after admission respectively. Shielding of the gonads in males during the X ray procedure may interfere with visualization of the pubic bone. Radioisotopic delineation of osteomyelitis may be possible earlier than conventional radiographs but Case 3 demonstrated that accumulation of the radioisotope in the bladder urine may interfere with the visualization of the bones of the pelvis.

Experience with three cases of acute primary osteomyelitis of the pubis has enabled us to summarize their salient features of presentation (Table 1).

Failure to detect osteomyelitis as the cause of illness may lead to inadequate therapy and thus predispose to chronic infection or other complications. Management should be in accordance with accepted standards for osteomyelitis (2, 7, 10, 14). Recovery of an organism preferably from the site of involvement should determine the choice of antibiotics. Organisms recovered were penicillin resistant *Staphylococcus aureus* by tissue aspiration in Case 1 and a group A beta hemolytic *Streptococcus* by blood culture in Case 3. All patients responded to the antibiotics chosen and recovery was satisfactory in each instance.

CONCLUSION

Osteomyelitis of the pubis while uncommon should be included in the differential diagnosis for patients presenting with symptoms suggestive of hip pathology. Our observations from these three reported cases suggest the following findings to be helpful:

- 1 Severe pain elicited on active motion of the hip but not on passive motion.
- 2 Absence of radiologic confirmation of hip involvement (including soft tissue signs).
- 3 Normal synovial fluid on hip aspiration.
- 4 Tenderness over the pubic area.

REFERENCES

- 1 Aksby R L & Staehle L T Pyogenic infections of the sacroiliac joint in children (Radioisotope bone

- scanning as a diagnostic tool) *Clin Orthop* 100 96 1974
- 2 Chalkin V II Management of severe forms of haematogenous osteomyelitis of the pelvic bone *Ortop Traumatol Prote* 27 29 1966 (Russ)
- 3 Chung S M K & Borns P Acute osteomyelitis adjacent to the sacroiliac joint in children (Report of 2 cases) *J Bone Joint Surg* 55A 630 1973
- 4 Dich V Q, Nelson J D & Hatalin K C Osteomyelitis of infants and children: A review of 163 cases *Am J Dis Child* 129 1273 1975
- 5 Dnan J J Two cases of primary osteomyelitis of the pubic bone *Can Med Assoc J* 41 436 1939
- 6 Edwards M S, Baker C J, Granberry W M & Barrett F F Pelvic osteomyelitis in children *Pediatrics* 61 6 1978
- 7 Ferguson A B Jr Osteomyelitis in children *Clin Orthop* 96 51 1973
- 8 Fournier A, Pauli A, Ducoulombier H, Cousin J & Huguet J Osteomyelitis of the pubic bone *J Sci Med Lille* 83 391 1967 (Fr)
- 9 Geist E S Osteomyelitis of pubic bone *JAMA* 77 1939 1971
- 10 Kulowski J, Hicks E O & Steindler A Operative treatment of suppurative lesions of the bony pelvis *Am J Surg* 79 405 1950
- 11 Letts R M, Aliff A & Sutherland J B Technetium bone scanning as an aid in the diagnosis of atypical osteomyelitis in children *Surg Gynecol Obstet* 140 899 1975
- 12 McWhorter G Osteomyelitis of the ischium and pubis *Surg Gynecol Obstet* 49 205 19 9
- 13 Morgan A & Yates A H The diagnosis of acute osteomyelitis of the pelvis *Postgrad Med J* 42 74 1966
- 14 Nelson J D The bacterial etiology and antibacterial management of septic arthritis in infants and children *Pediatrics* 50 437 1977
- 15 Scott L Osteomyelitis of the pelvis *South Med J* 20 104 1977
- 16 Waldvogel F A, Medoff G & Swartz M N Osteomyelitis: A review of clinical features, therapeutic considerations and unusual aspects (Part I) *N Engl J Med* 287 198 1976
- 17 Waldensky A O Osteomyelitis of the pelvic girdle *Arch Surg* 37 371 1938
- 18 Young F Acute osteomyelitis of the ilium *Surg Gynecol Obstet* 58 986 1934

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COMPARISONS BETWEEN SERUM CONCENTRATIONS OF THYROXINE AND THYROXINE BINDING PROTEINS IN SAMPLES SIMULTANEOUSLY OBTAINED FROM CAPILLARY PERIPHERAL VEIN CENTRAL VEIN AND AORTA IN NEWBORN INFANTS

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ABSTRACT Jacobsen B B and Peitersen B (University Clinic of Paediatrics Children's Hospital Fuglebakken Copenhagen Denmark) Comparisons between serum concentrations of thyroxine and thyroxine binding proteins in samples simultaneously obtained from capillary peripheral vein central vein and aorta in newborn infants *Acta Paediatr Scand* 68 43 1979 —A total number of 40 newborn infants with various maturity were studied 13 babies without perinatal events 19 infants recovered from transient diseases 6 infants with idiopathic respiratory distress syndrome and 2 infants with asphyxia indicating artificial ventilation Comparisons were performed between serum concentrations of thyroxine (T₄) thyroxine-binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) in capillary versus peripheral vein aorta versus central vein and finally in peripheral versus central veins In healthy infants serum T₄ concentrations in capillary blood and peripheral vein did not differ significantly Although serum concentrations of thyroid hormone-binding proteins tended to be increased in aortic compared to central venous specimens no statistically significant differences appeared In infants in good clinical conditions serum T₄ TBG TBPA and Alb levels were 6-8% higher in peripheral than in central veins possibly primarily due to a hemoconcentrating effect of venous stasis Therefore in evaluation of the thyroid variables in newborn infants the technique of blood sampling must be considered In most infants with idiopathic respiratory distress syndrome and in one asphyxiated baby a remarkable tendency to a low serum TBG and T₄ concentration in peripheral compared to central vein samples were observed

KEY WORDS thyroxine thyroxine binding globulin prealbumin albumin idiopathic respiratory distress syndrome neonates

Venous compression by arm tourniquet rises the filtration pressure across the capillary wall (8) and may increase total serum concentrations of thyroid hormones (16) Recently we demonstrated in adults that even shortterm venous stasis increases serum thyroxine and thyroxine binding protein levels by 7-11% (12) The capillary blood flow and the transcapillary escape of fluid and proteins are much higher in newborns than in adults (1 17 18 21 27) It has not yet been possible for technical reasons to perform similar investigations

of the influence of venous stasis in newborn infants

In newborn babies with low birth weights or idiopathic respiratory distress syndrome (IRDS) low serum thyroxine and thyroxine binding protein concentrations have been observed (3 11 13 14 23) These findings—also of relevance in screening for congenital hypothyroidism (7)—have not been fully explained

The present study was conducted in neonates with various maturity to compare serum concentrations of thyroxine and thyroxine

Table 1 Clinical data in 40 newborn infants aged 1 to 4 days

The groups of infants—see text

Group of infants	Birth weights (g)	Gestational age (weeks)	No. of infants		
			Fullterm	Premature	Small for date
I	2 960 (1 890–3 960)	38 (33–41)	7	3	3
II	2 450 (1 520–4 100)	39 (31–40)	7	4	8
III	2 585 (1 050–3 650)	35 (28–43)	3	4	1

binding proteins in samples obtained simultaneously from different parts of the vascular system: capillaries, peripheral veins after application of arm tourniquet and from central veins and aorta.

MATERIAL AND METHODS

A total number of 40 newborn infants aged 1 to 4 days were included in the study. All were euthyroid with serum thyrotropin concentrations within normal range (11). Maturity and gestational age were assessed as previously reported (10). From clinical conditions the infants were divided in three groups (Table 1).

Group I were healthy infants without perinatal asphyxia.

Group II were infants in clinical good condition with normal acid base status recovered from transient hypoglycemic, hypocalcemic or respiratory problems which had indicated umbilical catheterization.

Group III consisted of six infants with idiopathic respiratory distress syndrome and one premature and one postmature baby with severe asphyxia indicating intubation, artificial ventilation and application of umbilical catheters. All infants had stabilized circulation and normal body temperature when the study was performed.

Argyle® umbilical catheters of size 31 or 5 were used. The artery catheters were placed with the tip in the descending aorta at the level of 10th to 12th thoracic vertebra. The umbilical vein catheter tips were usually placed in the umbilical vein but in two infants the tip was in the portal sinus. Only in one infant the catheter tip was situated in the right atrium. No complications in relation to the catheterization were seen. Parents were informed and consent obtained. At least two blood samples were obtained simultaneously from each infant. The amount of blood taken from each infant did not exceed 5–6 ml. Capillary blood was drawn after heel prick and peripheral venous samples were obtained from a cubital vein after application of a tourniquet of rubber on the upper arm. Blood samples from the central venous system and aorta were taken from the umbilical catheters after withdrawing 2 ml blood prior to each sampling. This sample was re-

injected after the test sample had been obtained. Blood was centrifuged and serum frozen at -20°C until analyses were performed. All analyses were performed in duplicate.

Serum total thyroxine (T_4) was measured using a competitive binding microtechnique as previously reported (10). The intraassay coefficient of variation was 4% in the range of 77–129 nmol/l and 6–10% outside that range. Serum thyroxine binding globulin (TBG) was measured using rocket immunoelectrophoresis and autoradiography (14). The intraassay coefficient of variation was 4%. Serum prealbumin (TBPA) and albumin (Alb) were determined by immunoelectrophoresis as reported elsewhere (14). The intraassay coefficients of variation for both protein assays were 3%. The serum concentrations of thyroid hormone binding proteins were presented in terms of those in a reference serum pool from male adults and stated to 100 arbitrary units per liter. Using routine laboratory methods the absolute serum TBG, TBPA and Alb concentrations in the reference serum are 9.3 mg/l, 97 mg/l and 43 000 mg/l respectively (14). Normal ranges of serum T_4 , TBG, TBPA and Alb in newborn infants have been reported previously (11, 14).

In the statistical analyses the Wilcoxon's test for pair differences, the Spearman rank correlation coefficient R and the chi square (χ^2) test with Yates' correction were used (25).

RESULTS

The investigations were tripartite. Only serum concentrations in simultaneously obtained blood samples were compared.

Serum T_4 in capillary versus peripheral venous samples

In 13 healthy infants (group I) with various maturity a comparison was performed between serum T_4 concentrations in capillary versus in peripheral venous blood. In capil-

Table 2 Serum thyroxine (T_4) thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) concentrations in blood samples obtained simultaneously from aorta and central venous system

The groups of infants—see text

Groups of infants		Serum T_4 (nmol/l)		Serum TBG (arb u/l)		Serum TBPA (arb u/l)		Serum Alb (arb u/l)	
		Aorta	Central vein	Aorta	Central vein	Aorta	Central vein	Aorta	Central vein
II	Median	15	14	191	168	78	27	77	80
	Range	(11–75)	(116–781)	(111–734)	(113–739)	(25–37)	(23–37)	(65–86)	(53–89)
	No	8	7	6	6	6	7	7	7
		$p > 0.1$		$p > 0.1$		$p > 0.1$		$p > 0.1$	
III	Median	192	176	138	133	73	77	78	74
	Range	(64–134)	(67–147)	(10–06)	(94–05)	(17–30)	(17–28)	(64–95)	(60–91)
	No	6	6	6	6	6	6	6	6
		$p > 0.1$		$p > 0.1$		$p > 0.1$		$p > 0.1$	

p is the level of significance for the Wilcoxon test for pair differences

lary blood serum T_4 ranged 93–208 nmol/l (median 167 nmol/l). In venous samples serum T_4 ranged 93–212 nmol/l (median 154 nmol/l) which did not differ significantly from those in capillary blood ($p > 0.1$). The serum T_4 concentrations but not the individual differences between capillary and venous concentrations were influenced by maturity and postnatal age. Measurement of thyroxine binding proteins was not performed because an adequate amount of serum was not available.

Serum T_4 , TBG, TBPA and Alb in samples from umbilical vessel catheters

In 14 infants from group II and III blood samples were drawn simultaneously from aorta and the central venous system. Table 2 shows median and range of the serum T_4 , TBG, TBPA and Alb concentrations. In the four infants with idiopathic respiratory distress syndrome serum T_4 and TBG levels were lower than those of other neonates whereas in the two asphyxiated infants serum T_4 and TBG

Table 3 Serum thyroxine (T_4) thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) concentrations in specimens from peripheral (P V) and central veins (C V) obtained simultaneously

The groups of infants—see text

Groups of infants		Serum T_4 (nmol/l)		Serum TBG (arb u/l)		Serum TBPA (arb u/l)		Serum Alb (arb u/l)	
		P V	C V	P V	C V	P V	C V	P V	C V
II	Median	184	175	164	153	76	74	76	73
	Range	(85–317)	(180–781)	(171–734)	(119–77)	(17–35)	(15–37)	(49–101)	(55–88)
	No	19	16	16	17	17	18	18	18
		$p < 0.05$		$p < 0.01$		$p < 0.01$		$p < 0.01$	
III	Median	108	111	13	136	76	4	79	74
	Range	(75–176)	(6–147)	(81–708)	(85–705)	(19–9)	(17–78)	(63–98)	(69–91)
	No	7	7	7	7	8	7	7	7
		$p > 0.1$		$p > 0.1$		$p > 0.1$		$p > 0.1$	

p is the level of significance for the Wilcoxon test for pair differences

were comparable to those in healthy infants with similar birth weights. Although serum protein concentrations tended to be higher in aortic than in venous samples, no statistically significant differences were observed. The individual differences between serum concentrations in aorta and central veins were not related to maturity, birth weight or disease.

Serum T_4 , TBG, TBPA and Alb in samples from peripheral versus central veins

In 22 infants a comparison between serum T_4 , TBG, TBPA and Alb level in peripheral versus central veins was performed. Furthermore, in 5 infants serum T_4 and T_4 binding protein concentrations in peripheral veins were compared with those in aorta and the results were included in the calculations (Table 3). This does not influence the conclusions, because serum concentrations in aorta and central veins are comparable.

Table 3 shows the median and range values of serum T_4 , TBG, TBPA and Alb in 19 infants in a good clinical condition (group II) and in 6 infants with IRDS and 2 asphyxiated babies (group III). In group II infants serum concentrations of T_4 and T_4 binding proteins were significantly higher in peripheral than in central veins (and aorta). The median increases were 6–8% and a statistically significant correlation was found between increases in serum T_4 and serum TBG concentrations ($R\ 0.51$, $p < 0.05$).

In infants in group III serum TBPA and serum Alb levels were also higher in peripheral than in central veins (although not statistically significant). Serum T_4 and TBG, in contrast, tended to be lower in the peripheral vein. In fact, serum TBG concentrations in peripheral veins were significantly more often reduced in infants with IRDS than in the other infants studied ($\chi^2\ 4.01$, $p < 0.05$), whereas this was not statistically significant concerning serum T_4 ($\chi^2\ 1.08$, $p > 0.1$).

Median values of serum T_4 and TBG concentrations (Table 3) in both peripheral and

central veins were lower in group III than in group II infants.

DISCUSSION

Previous investigations have demonstrated a reasonably good correlation between T_4 concentrations in dried capillary blood (eluted from filter paper, recovery 74–95%) and in whole blood or plasma (4, 20, 28), but it was noticed that in infants capillary serum T_4 samples may be variably unreliable (28). In the present study, however, capillary blood (after heel prick) was sampled in glasses immediately centrifuged and serum frozen until analysis was performed. Using that technique, serum T_4 concentrations in capillary and peripheral venous blood did not differ significantly, neither in full-term nor in low birth weight newborns. The discrepancy between previous and present studies seems to be due to different methodology.

The present finding of comparable serum T_4 , TBG and Alb concentrations in samples from umbilical artery and vein catheters seems of some importance. Ewerbeck & Levens (6) and Tulzer (26) measured a higher serum Alb and a lower alpha globulin concentration in umbilical artery than in umbilical vein specimens. Later Rose et al. (24) have reported that protein bound iodine concentrations in umbilical vein and artery specimens were of the same magnitude, which does agree with our results. These earlier investigations were performed using samples from a clamped umbilical cord at the delivery, which might involve measurement of placental proteins. Determinations of serum T_4 and T_4 binding protein concentrations in the central arterial and venous system of human newborns have not previously been reported. The samples from the central venous system consisted mainly of blood from the portal vein. Due to a sphincter function of ductus venosus (15), probably only a small amount of blood was coming from the inferior caval vein. The tendency towards higher serum protein concentrations in aorta compared

o central veins may be due to the hepatic protein synthesis

The most important observation in the present study may be the finding in group II infants of higher serum concentrations of T_4 and T_4 binding proteins in peripheral vein specimens compared to those in blood from the central vascular system obtained simultaneously. For the infants in a clinical good condition serum concentrations of T_4 , TBG, TBPA and Alb were 6-8% higher in peripheral vein samples and a statistically significant correlation was found between the increase in serum T_4 and in serum TBG. These findings might be explained by a hemoconcentrating effect induced by peripheral venous stasis.

In adults peripheral venous stasis causes a transudation of proteinfree solute from the circulation with a resultant rise in plasma protein concentrations (2, 8). Circulating thyroid hormones are almost totally bound to serum protein (22) and it is known that in adults venous compression by arm tourniquet may increase serum concentrations of thyroid hormones by 7-15% (16). Recently we demonstrated that such increases in serum T_4 as well as in serum TBG, TBPA and Alb concentrations occur after short-term (2-3 min) venous stasis (12). We have not been able to obtain a sufficient amount of blood in similar investigations in newborn infants.

During the early neonatal period of life the transcapillary movements of fluids (1, 17, 27) and proteins are increased compared to later in infancy. In fact the transcapillary escape rate of Alb is 3-4 times higher in the newborn mature infants than in adults (18, 21) which might also apply to the other T_4 binding proteins (9). The transcapillary escape and blood flow is even higher in premature than in mature newborns (1, 17). These observations however were not reflected in the present results possibly because recirculation of fluids and proteins to the vascular system is also increased during the neonatal period.

In infants with IRDS or asphyxia the peripheral blood flow is reduced and the vascular

resistance increased (1, 17) possibly due to increased catecholamine release (19). Further more changes in blood pressure were probable in group III patients due to the intubation and artificial ventilation which might contribute to the explanation of the individual differences observed. Our findings of lower median serum T_4 and TBG concentrations in infants with IRDS than in the other infants with comparable birth weights are in accordance with previous investigations (3, 13, 23). Serum TBG was more often reduced in peripheral veins as compared to central venous specimens in infants with IRDS than in other infants. Further confirmations are clearly needed before any conclusions might be drawn.

Previous investigations have demonstrated the significance of gestational age, birth weight, postnatal age and diseases for serum levels of T_4 , TBG, TBPA and Alb in newborn infants (3, 5, 11, 13, 14, 23). The present study indicates that the site of blood sampling must also be considered.

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REFERENCES

1. Celander O. Studies of the peripheral circulation. In D. E. Cassels (ed.) *The heart and circulation in the newborn infant*. Grune & Stratton, New York, 1966, p. 98.
2. Christiansen C, Næstoft J, Hvidberg E, Larsen N E & Petersen B. An easy procedure for in vivo estimation of protein binding and correction of elevated serum values induced by venous stasis. *Clin Chem Acta* 62: 65, 1975.
3. Cuestas R A, Lundall A & Engel R. Low thyroid hormones and respiratory distress syndrome of the newborn. Studies on cord blood. *N Engl J Med* 295: 298, 1976.
4. Dusault J H & Laberge C. Dosage de la thyroxine par méthode radio-immunologique dans l'éluat de

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SERUM CONCENTRATIONS OF THYROXINE BINDING GLOBULIN PREALBUMIN AND ALBUMIN IN HEALTHY FULLTERM SMALL FOR GESTATIONAL AGE AND PRETERM NEWBORN INFANTS

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ABSTRACT Jacobsen B B Peetersen B Andersen H J and Hummer L (The University Clinic of Paediatrics Children's Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for-gestational age and preterm newborn infants Acta Paediatr Scand 68 49 1979—Simultaneous serum concentrations of thyroxine-binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) were measured in 130 fullterm 32 small for-gestational age and 25 preterm infants during their first six days of life. In all infants serum concentrations of TBG were higher and serum TBPA and Alb were lower than in male adults. Even higher serum TBG levels were found in the mothers. There was no correlation between serum concentrations in paired maternal and cord sera. In infants with birth weights appropriate for gestation serum TBG TBPA and Alb concentrations increased progressively with gestational age. In small for-gestational age infants born at term serum concentrations of TBG and Alb were lower than those in full term but higher than those in premature newborns. Serum TBPA in small for-gestational age babies was even lower than seen in premature. A positive correlation was found between thyroid hormones and TBG concentrations not between serum TBPA and thyroid hormones. The ratios between serum concentration of thyroid hormones and proteins might indicate that more thyroid hormone-binding sites are occupied in fullterm than in low birth weight newborns. However the main reason for the different serum levels of thyroid hormones in fullterm small for-gestational age and preterm babies is probably the various serum TBG concentrations demonstrated in these infants.

KEY WORDS Thyroxine binding globulin prealbumin albumin SGA infants preterm infants

Thyroid hormones in human serum are mainly bound to thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) (3, 14, 23). It has been shown that serum thyroxine concentration in fetuses (7) and in cord blood (20) increases with gestational age. This relationship seems—at least in part—to be caused by variation in serum TBG binding capacity (1, 7). However, Oddie et al. (20) did not find a correlation between gestational age and TBG concentration in cord sera from infants of 30 to 45 weeks gestation. No data on any small for gestational age infants were given.

Recently we reported that serum concen-

trations of thyroxine (T_4) and triiodothyronine (T_3) are lower in small for gestational age (SGA) babies born at term than in mature full term (FT) newborns, even lower serum concentrations were seen in preterm (PT) infants with birth weights appropriate for gestation (12).

The present investigations were undertaken to measure the serum concentrations of TBG TBPA and Alb in healthy newborn infants of various maturity and gestational age and to study the relationship between the protein and thyroid hormone concentrations in these babies.

- sang séché Nouvelle méthode de dépistage de l'hypothyroïdie néonatale *Union Med Canada* 107 2062 1973
- 5 Erenberg A Phelps D L Lam R & Fisher B A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics* 53 211 1974
 - 6 Ewerbeck V H & Levens H E Über die Bildung der Serumeiweißkörper in der Placenta sowie ihren Beitrag und Fettstoffwechsel des Feten *Monatsschr Kinderheilkd* 99 297 1951
 - 7 Fisher D A Burrow G N Dussault J H Hollingsworth D R Larsen P R Man E H & Wal-fish P G Recommendations for screening programs for congenital hypothyroidism Report of a committee of the American Thyroid Association *J Pediatr* 89 692 1976
 - 8 Gerbrandy J Leewen A M V Hellendoorn H B A & Vries L A The binding between electrolytes and serum proteins calculated from and in vivo filtration method *Clin Sci* 19 181 1960
 - 9 Irvine C H G & Simpson Morgan M W Relative rates of transcapillary movement of free thyroxine protein bound thyroxine thyroxine binding proteins and albumin *J Clin Invest* 54 156 1974
 - 10 Jacobsen B B Andersen H Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand* 66 541 1977
 - 11 Jacobsen B B Andersen H J Peitersen B Dige Petersen H & Hummer L Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Paediatr Scand* 66 681 1977
 - 12 Jacobsen B B Petersen B & Andersen H Changes in serum levels of thyroxine and thyroxine binding proteins (TBG TBPA Albumin) induced by venous stasis *Acta Endocrinol (Abh)* 85 39 1977
 - 13 Jacobsen B B Peitersen B & Andersen H J Serum concentrations of thyroid hormone and thyroid hormone binding proteins in infants with respiratory distress syndrome 16th annual meeting of European Society for Pediatric Endocrinology Cambridge England 1977
 - 14 Jacobsen B B Peitersen B Andersen H J & Hummer L Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for gestational age and preterm newborn infants *Acta Paediatr Scand* 1978 accepted for publication
 - 15 Jegier W Blankenship W & Lind J Venous pressure in the first hour of life and its relationship to placental transfusion *Acta Paediatr Scand* 52 485 1963
 - 16 Judd S J Carter J N & Corcoran J M Circulating thyroid hormone concentrations and postural and venous compression *Br Med J* IV 735 1975
 - 17 Kidd L Levison H Gemmel P Aharon A & Swyer P R Limb blood flow in normal and sick newborn *Am J Dis Child* 112 402 1966
 - 18 Krasimkoff P A Albumin metabolism in normal mature and premature children FADL Copenhagen 1975 Thesis
 - 19 Lagerkranz H & Bistoletti P Catecholamine release in the newborn infant at birth *Pediatr Res* 11 889 1973
 - 20 Larsen P H & Brodsky A Thyroxine immunoassay using filter paper blood samples for screening of neonates for hypothyroidism *Pediatr Res* 9 604 1975
 - 21 Parving H H Klebe J G & Ingemar C J Simultaneous determination of plasma volume and transcapillary escape rate with ¹²⁵I labeled albumin and T 1824 in the newborn *Acta Paediatr Scand* 67 248 1973
 - 22 Prince H P & Ramsden D B A new theoretical description of the binding of thyroid hormones by serum proteins *Clin Endocrinol* 7 307 1977
 - 23 Redding W A & Pereira C Thyroid function in respiratory distress syndrome of the newborn *Pediatrics* 54 423 1974
 - 24 Rowe H Russel K P & Starr P Serum protein bound iodine of mothers and infants at delivery in premature and term pregnancies *Am J Obstet Gynecol* 86 767 1963
 - 25 Siegel S *Non parametric statistics for the behavioral sciences* McGraw Hill Kogakusha Ltd Tokyo 1956
 - 26 Tulzer H Über Beziehungen zwischen Placenta und fötalen Serumeiweißbild *Arch Gynakol* 197 1 1959
 - 27 Usher M Shephard M & Lind J The blood volume of the newborn infant and placental transfusion *Acta Paediatr Scand* 52 497 1963
 - 28 Wal-fish P G Screening for neonatal hypothyroidism using dried capillary blood method Observations on methodologic factors selection criteria and preliminary results In D A Fisher & G N Burrow (eds) *Perinatal thyroid physiology and disease* Raven Press Publ New York 1975 p 239

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SERUM CONCENTRATIONS OF THYROXINE BINDING GLOBULIN PREALBUMIN AND ALBUMIN IN HEALTHY FULLTERM SMALL FOR GESTATIONAL AGE AND PRETERM NEWBORN INFANTS

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ABSTRACT Jacobsen B B Peetersen B Andersen H J and Hummer L (The University Clinic of Paediatrics Children's Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark): Serum concentrations of thyroxine binding globulin prealbumin and albumin in healthy fullterm small for-gestational age and preterm newborn infants *Acta Paediatr Scand* 68 49 1979.—Simultaneous serum concentrations of thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) were measured in 130 fullterm 32 small for-gestational age and 25 preterm infants during their first six days of life. In all infants serum concentrations of TBG were higher and serum TBPA and Alb were lower than in male adults. Even higher serum TBG levels were found in the mothers. There was no correlation between serum concentrations in paired maternal and cord sera. In infants with birth weights appropriate for gestation serum TBG TBPA and Alb concentrations increased progressively with gestational age. In small for-gestational age infants born at term serum concentrations of TBG and Alb were lower than those in full term but higher than those in premature newborns. Serum TBPA in small for-gestational age babies was even lower than seen in premature. A positive correlation was found between thyroid hormones and TBG concentrations, not between serum TBPA and thyroid hormones. The ratios between serum concentration of thyroid hormones and proteins might indicate that more thyroid hormone-binding sites are occupied in fullterm than in low birth weight newborns. However, the main reason for the different serum levels of thyroid hormones in fullterm small for gestational age and preterm babies is probably the various serum TBG concentrations demonstrated in these infants.

KEY WORDS Thyroxine binding globulin prealbumin albumin SGA infants preterm infants

Thyroid hormones in human serum are mainly bound to thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) (3, 14, 23). It has been shown that serum thyroxine concentration in fetuses (7) and in cord blood (20) increases with gestational age. This relationship seems—at least in part—to be caused by variation in serum TBG binding capacity (1, 7). However, Oddie et al. (20) did not find a correlation between gestational age and TBG concentration in cord sera from infants of 30 to 45 weeks gestation. No data on any small for gestational age infants were given.

Recently we reported that serum concen-

trations of thyroxine (T_4) and triiodothyronine (T_3) are lower in small for gestational age (SGA) babies born at term than in mature full term (FT) newborns, even lower serum concentrations were seen in preterm (PT) infants with birth weights appropriate for gestation (12).

The present investigations were undertaken to measure the serum concentrations of TBG, TBPA and Alb in healthy newborn infants of various maturity and gestational age and to study the relationship between the protein and thyroid hormone concentrations in these babies.

Table 1 Clinical data of fullterm (FT) small for gestational age (SGA) and preterm (PT) infants aged 0 to 6 days

Infants	No of infants (n)	Gestational age (weeks)		Birth weights (g)	
		Median	Range	Median	Range
FT	130	39	(37-42)	3 500	(2 800-4 500)
SGA	32	39	(37-43)	2 450	(1 520-2 900)
PT	25	34	(25-36)	2 185	(1 570-2 750)

MATERIAL AND METHODS

A total number of 187 healthy neonates of 25 to 43 weeks gestation were included in the study (Table 1). Gestational age and maturity were assessed as reported elsewhere (11, 12). All patients with respiratory distress syndrome or asphyxia (Apgar score ≤ 6 after 1 min) were excluded. Postnatal age ranged from 0 to 6 days. All the PT infants had birth weights appropriate for gestation. The birth weights differed significantly in the three groups of new borns ($p < 0.001$). Low birth weight infants were usually placed in incubators. All infants were fed with human milk.

Usually blood was drawn from a peripheral vein. Cord blood was taken from 36 babies at birth. Only one blood sample was obtained from each infant. The parents were informed and consent obtained. Venous blood was also obtained from 20 mothers at the delivery. Blood was centrifuged immediately and serum frozen at -20 until analyses were performed. All analyses were done in duplicate. The thyroid hormone and thyrotropin concentrations in sera from these infants and mothers have previously been reported by us (12).

The thyroid hormone binding protein concentrations were measured using Laurell's rocket immunoelectrophoresis (16) in 1% (w/v) antibody containing agarose gel 2-3 V/cm for 15 hours at 15 in Trisbarbitone buffer pH 8.6. Antibodies against TBG, TBPA and Alb were obtained from Dakopatts Copenhagen, Denmark. When measuring serum TBPA and serum Alb the rockets were visualized by staining the gel with Coomassie Brilliant Blue. The within assay coefficient of variation was 2% and the between assay coefficient of variation was 3% for both assays. The antibody against TBG was not mono-specific; after preincubation of the sera with 125 I thyroxine (Amersham, England, 200 μ Ci/4.3 μ g/ml) the precipitate of TBG was visualized by autoradiography (5 days) as reported by Nielsen et al. (19). The within assay coefficient of variation for the TBG assay was 3% and the between assay coefficient of variation was 4%.

The results were presented in terms of those obtained in a reference serum. The reference serum was a serum pool from 100 apparently healthy male blood donors and the TBG, TBPA and Alb concentrations in this serum were arbitrarily set to 100 units per liter. Using routine laboratory methods the absolute concentrations of TBPA and Alb in the reference serum are 297 mg/l and 43 000 mg/l respectively. The absolute TBG concentration measured by radioimmunoassay is 9.3 mg/l.

In the statistical analyses the Kruskal-Wallis test, the Mann-Whitney test and Spearman rank correlation coefficient (R) were used (25).

RESULTS

Table 2 lists median value and interquartile range of serum TBG, TBPA, Alb and thyroid hormone concentrations in the different groups of neonates. The protein concentrations were significantly higher in FT than in low birth weight infants. Furthermore, serum TBG and serum Alb levels were higher and serum TBPA lower in SGA compared with those in PT newborns.

The serum TBG concentrations in infants and mothers were much higher and serum TBPA and Alb lower than in the reference serum (Table 2). The serum TBG in mothers was significantly higher than in infants ($p < 0.01$). The serum concentrations of thyroid hormone binding proteins were studied in 20 paired maternal and cord sera. The correlation coefficient R for serum TBG in mothers versus serum TBG in infants was 0.11, the corresponding correlation coefficients for serum TBPA and serum Alb were 0.27 and 0.31 respectively. Thus no significant correlations were seen ($p > 0.1$).

The serum levels of thyroid hormone binding proteins in the neonates (Fig. 1) did not change significantly during the first week of life. However, some low birth weight infants aged 5-6 days had higher serum values than

¹ Kindly performed by J. Kohrle, D.Sc., Department of Endocrinology, University of Hannover, West Germany.

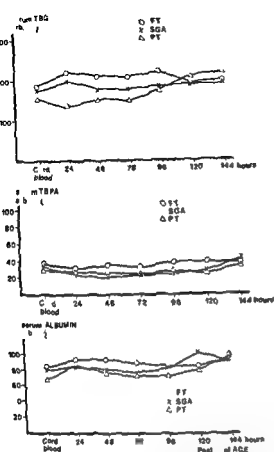


Fig 1 Variation of serum thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin concentrations with postnatal age in full-term (FT) small for gestational age (SGA) and preterm (PT) newborn infants. Median values of protein concentration for each age group are shown. No statistically significant postnatal changes were observed. (Arb u/l is concentration in term of values in a reference serum (a serum pool from male adults which is stated to 100 arbitrary units per liter absolute concentrations—see text))

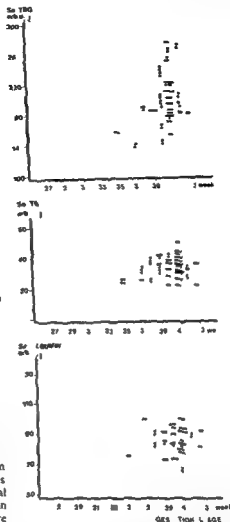


Fig 2 Variation of serum thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin concentrations with gestational age in full-term (FT) small for gestational age (SGA) and preterm (PT) newborn infants (Arb u/l—see legend to Fig 1)

the other infants during the first postnatal days. Differences in birth weights might be one reason for this because serum protein concentrations seem to increase with birth weight. The correlation coefficients (R) for TBG versus birth weight, TBPA versus birth weight and Alb versus birth weight were 0.30, 0.30 and 0.20 respectively ($p < 0.01$). Although statistically significant, the correlations were obviously not very strong.

Fig 2 shows serum TBG, serum TBPA and serum Alb concentrations in infants with birth

weights appropriate for gestation plotted versus gestational age in weeks. The correlation coefficients (R) for TBG versus gestational age were 0.43, 0.26 and 0.22 respectively ($p < 0.003$). Thus the correlations of serum proteins with gestational age were of the same magnitude as with birth weights.

In the group of SGA infants, serum concentrations of thyroid hormone binding proteins did not show any correlation with birth weights or gestational age.

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um T₃ (nmol/l)

Median	Interquartile range
	(1.57-3.11)
	(1.35-2.33)
	(0.68-1.45)
0.01	
	(1.95-3.75)

ratios were significantly different in the three groups of newborn infants the highest values appeared in FT and the lowest values were seen in PT babies. Similar results were obtained concerning T₄/Alb and T₃/Alb ratios. The ratios between serum T₄ and TBG concentrations were significantly higher in infants than for maternal values ($p < 0.01$). This was less evident for infants between serum T₃ and TBG values.

DISCUSSION

The present study demonstrates that in newborn infants serum TBG concentration is higher and serum TBPA and serum Alb are lower than in male adults (i.e. in our reference serum). Furthermore our data confirm the findings that even higher serum TBG concentrations appear in the mothers at delivery (4, 5, 18, 24). However there was no correlation between serum concentrations of thyroid hormone binding proteins in paired cord and maternal sera indicating very little placental transfer of these proteins.

It has been shown that in human fetuses of 8 to 24 weeks gestation the serum binding capacity of TBG increases with gestational age corresponding to a relative decrease in binding capacity of TBPA and Alb (1, 7). Neverthe-

less in some earlier studies the serum binding capacity of TBG in premature babies has been found to be the same as in mature infants (17, 22). In a recent study by Oddie and associates the cord blood TBG concentrations did not vary significantly with gestational age (20). In that study serum TBG was measured by radioimmunoassay the gestational age of the infants ranged from 30 to 45 weeks and it was suggested that most of the increase in TBG in fetal serum occurs prior to 30 weeks gestation (20). We found in newborns with birth weights appropriate for gestation that serum concentrations of TBG, TBPA and Alb increased progressively with gestational age. In the group of infants who were small for gestational age the serum concentration of thyroid hormone binding proteins did not show any correlation with birth weight or gestational age probably because that group was very heterogeneous concerning birth weights in relation to gestational age. One reason for the differences in the results of previous investigations (17, 20, 22) and the present study could be that we made a distinction between FT and SGA newborns. Different methodology and various gestational age of the infants might also influence the results. In a study of serum concentrations of total protein and albumin Bergstrand et al. (2) found lower serum concentrations in SGA than in FT but higher values than in PT neonates findings which are in accordance with our data. The present finding of a lower serum TBPA level in SGA than in other groups of newborns has not been recorded previously and needs further investigations.

The causes of different serum levels of thyroid hormone binding proteins in infants with various gestational age and maturity are unknown. The increase in serum levels in infants with birth weights appropriate for gestation might be due to a progressive increase in protein synthesis with gestation. This does not explain the lower serum protein levels in SGA compared to FT neonates. These are comparable to finding of decreased thyroid hormone

Table 2 Serum concentrations of thyroxine binding globulin (TBG) prealbumin (TBPA) albumin (Alb) and thyroid hormones in newborn babies aged 0-6 days and in mothers at delivery

Abbreviations—see Table 1

	Serum TBG (arb u/l)		Serum TBPA (arb u/l)		Serum Alb (arb u/l)		Serum T ₄ (nmol/l)	
	Median value	Inter quartile range	Median value	Inter quartile range*	Median value	Inter quartile range*	Median value	Inter quartile range*
Infants								
FT (n=130)	200	(182-227)	36	(30-40)	88	(81-93)	236	(180-278)
SGA (n=32)	175	(150-204)	23	(21-32)	81	(76-89)	193	(118-250)
PT (n=25)	154	(139-176)	26	(22-29)	77	(65-90)	145	(119-184)
Kruschal Wallis test	p<0.001		p<0.001		p<0.001		p<0.001	
Mothers (n=20)	277	(260-301)	71	(59-88)	80	(70-84)	175	(178-240)

* Interquartile range signifies the range between the quartile of the lowest values and the quartile of the highest (i.e. fifty per cent of the distribution)

In infants with birth weights appropriate for gestation (i.e. FT and PT) serum TBG was correlated with serum Alb (R 0.39 p <0.001), but not correlated with serum TBPA (R 0.04). Comparable observations were made in SGA newborns.

The thyroid hormone binding protein levels in sera from FT, SGA and PT neonates differed in the same way as the thyroid hormone levels (Table 2) and investigations of the relation between these variables were therefore performed. Only infants two to six days of age were included in the calculations because pronounced changes in total and free thyroid hormone concentrations appear during the first 24 hours after birth (6, 12). A good correlation was found in all groups of infants between

thyroid hormone and TBG concentration. The correlation coefficients for serum T₄ versus serum TBG were 0.68, 0.57, and 0.60 for FT, SGA and PT infants (p <0.001). The corresponding values for serum T₃ versus serum TBG were 0.35, 0.54 and 0.58 respectively (p <0.02). There was no correlation between thyroid hormone and TBPA concentrations in any groups. In FT and SGA infants but not PT babies the thyroid hormone seemed to be related to Alb concentrations but the correlations were less strong (R 0.36 to 0.45).

In an attempt to evaluate the saturation of hormone binding sites the individual serum T₄ and serum T₃ were divided with corresponding serum TBG concentrations, median values as shown in Table 3. Both T₄/TBG and T₃/TBG

Table 3 Median values of ratios between serum concentrations of thyroid hormones and thyroxine binding globulin and albumin in infants 2 to 6 days of age and in mothers at delivery

Abbreviations—see Table 1

Ratio $\times 10^2$	Newborn babies			Kruschal Wallis test	Mothers (n=20)
	FT (n=92)	SGA (n=26)	PT (n=19)		
T ₄ /TBG	118	112	95	p <0.05	64
T ₄ /Alb	270	237	225	p <0.05	233
T ₃ /TBG	1.09	1.07	0.69	p <0.001	0.79
T ₃ /Alb	2.67	2.47	1.65	p <0.001	2.90

- small for gestational age infants *Acta Paediatr Scand* 61 18 1972
- 3 Bernal J & Refetoff S The action of thyroid hormone *Clin Endocrinol* 6 27 1977
 - 4 De Nayer P Malvaux P Van Den Schneck H G Beckers C & De Visscher M Free thyroxine in maternal and cord blood *J Clin Endocrinol* 6 233 1966
 - 5 Dussault J Row V V & Lickish G Studies of serum triiodothyronine concentration in maternal and cord blood Transfer of triiodothyronine across the human placenta *J Clin Endocrinol* 29 595 1969
 - 6 Erenberg A Phelps D L Lam R & Fisher H A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics* 57 111 1974
 - 7 Greenberg A H Czernushow P Reba H C Tyson J & Blizzard R M Observations on the maturation of thyroid function in early fetal life *J Clin Invest* 49 1790 1970
 - 8 Hesch R D Gatz J Juppner H & Stubbe P TBG-dependency of age related variations of thyroxine and triiodothyronine *Horm Metab Res* 9 141 1977
 - 9 Ingelbleek Y De Visscher M & De Nayer P Measurement of prealbumin as index of protein-calorie malnutrition *Lancet* II 106 1972
 - 10 Ingelbleek Y De Nayer P & De Visscher M Thyroxine binding globulin in infant protein-calorie malnutrition *J Clin Endocrinol Metab* 39 178 1974
 - 11 Jacobsen B B Andersen H J Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand* 66 341 1977
 - 12 Jacobsen B B Andersen H J Petersen H Dige Petersen H & Hummer L Serum levels of thyrotropin thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Paediatr Scand* 66 681 1977
 - 13 Koivisto M Simola S Haahtela J & Reinila M Thyroid function assay using the effective thyroxine ratio in infancy *Scand J Clin Lab Invest* 33 105 1974
 - 14 Koreck L & Tabachnick M Thyroxine protein interactions *J Biol Chem* 251 3558 1976
 - 15 Krasulnikoff M A Albumin metabolism in normal mature and premature children FADL Copenhagen 1975
 - 16 Laurell C B Quantitative estimations of proteins by electrophoresis in agarose gels containing antibodies *Anal Biochem* 15 45 1966
 - 17 Lemarchand Beraud T Genazzani A R Bagnoli F & Casoli M Thyroid function in the premature and the fullterm newborn *Acta Endocrinol (Abh)* 70 445 1977
 - 18 Montalvo J M Wahner H W Mayberry W E & Lum E K Serum triiodothyronine total thyroxine and thyroxine to triiodothyronine ratios in paired maternal cord sera and at one week and one month of age *Pediatr Res* 7 706 1973
 - 19 Nielsen H G Buus O & Weeke M A rapid determination of thyroxine binding globulin in human serum by means of the Laurell rocket immunoelectrophoresis *Clin Chim Acta* 36 133 1972
 - 20 Oddie T H Fisher D A Bernard B & Lam R W Thyroid function at birth in infants of 30 to 45 weeks gestation *J Pediatr* 90 803 1977
 - 21 Pedersen K O A systematic study of variables affecting protein binding of thyroxine and triiodothyronine in serum *Scand J Clin Lab Invest* 34 47 1974
 - 22 Perry R E Hodgman J E & Starr P Maternal cord and serial venous blood Protein bound iodine thyroid binding globulin thyroid binding albumin and prealbumin in premature infants *Pediatrics* 35 759 1965
 - 23 Robbins J Structure and function of thyroxine transport proteins In I H Dysdale R Ramsdan & H Hoffenberg (eds) *Thyroid hormone metabolism* Academic Press London 1975
 - 24 Robin N I Refetoff S Fang V & Selenkow H A Parameters of thyroid function in maternal and cord serum at term pregnancy *J Clin Endocrinol Metab* 29 1779 1969
 - 25 Siegel S Nonparametric statistics for the behavioral sciences McGraw Hill Kogakusha Tokyo 1956

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binding levels in sera from protein-calorie malnutrition (9-10). It remains to clarify whether a lower protein synthesis or higher catabolism occur in SGA in comparison with other newborn infants. Other explanations may also be considered. Karlinikoff (15) has reported that the lower serum Alb concentration in PT babies might be explained by dilution of the total intravascular mass of albumin in premature babies because the total intravascular mass expressed per kg body weight were equal in the PT and FT newborns. However, the different serum protein levels in the present study can not exclusively be caused by dilution which would change the protein concentrations equally.

The TBG is physiologically the most important carrier of thyroid hormones in blood with an affinity for T_4 and T_3 which is much greater than the affinity of TBPA and Alb for these hormones (3, 14, 21, 23). This agrees with our finding of a very good correlation between serum concentrations of thyroid hormones and TBG in the newborns. Serum concentration of TBPA was very low and not correlated with serum TBG for which reason it was not surprising that no correlation was found between TBPA and thyroid hormone concentrations.

The increased serum TBG concentration found in all newborn infants is probably the reason why the postnatal rise in free serum T_4 and T_3 concentrations is transitory (6) in spite of a more sustained increase in total thyroid hormone concentrations which appears in neonates (6, 12, 17). The thyroid hormone binding proteins are serving as reservoirs of hormones. Previous *in vitro* studies have indicated that the thyroxine binding proteins are slightly more saturated in newborns than in mothers (13, 24). We confirmed the observations that the ratio between serum T_4 and TBG concentration is higher in infants than in adults (8). Furthermore, as shown in Table 3 the ratios between serum T_4 as well as serum T_3 and TBG or Alb concentrations were significantly different in the three groups of new-

borns. Although the number of infants aged 1 to 6 days in each group was rather small, our data might indicate that more binding sites are occupied per unit protein in FT than in SGA babies, even fewer binding sites are occupied in PT neonates. Differences in body temperature and intravascular pH might possibly influence the hormone bindings (21) and one assumption is therefore that the free hormone concentrations and the affinity of the proteins for thyroid hormones are the same in the three groups of newborns. In fact, Greenberg et al (8) reported that T_4 binding characteristics of fetal TBG and free thyroid hormone concentrations in fetuses of 24 weeks gestations were like those in newborns.

We have previously reported that the pituitary TSH responses to exogenous TRH as well as the effect of endogenous TSH measured by thyroid hormone increases was of the same magnitude in low birth weight infants as in fullterm (11). The serum concentrations of TBG (and Alb) differed in FT, SGA and PT neonates in the same way as the thyroid hormone levels and are probably the main reasons for the various thyroid hormone levels in these infants. Further investigations of the postnatal changes in hormone and serum protein concentrations are in progress.

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REFERENCES

- Andreoli M & Robbins J. Serum proteins and thyroxine protein interaction in early human fetuses. *J Clin Invest* 41: 1070-1962.
- Bergstrand C G, Karlsson B W, Lindberg T & Ekelund H. Alfafoetoprotein, albumin and total protein in serum from preterm and term infants and

PRESSURE PASSIVE CEREBRAL BLOOD FLOW AND BREAKDOWN OF THE BLOOD-BRAIN BARRIER IN EXPERIMENTAL FETAL ASPHYXIA

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ABSTRACT Lou H C Lassen N A Tweed W A Johnson U Jones M and Palahniuk R J (Department of Anaesthesia Health Sciences Centre Winnipeg Canada) Pressure passive cerebral blood flow and breakdown of the blood-brain barrier in experimental fetal asphyxia Acta Paediatr Scand 68 57 1979.—Cerebral blood flow (CBF) was studied in non-extubated near term sheep fetuses using the radioactive microsphere technique. By partially occluding the umbilical vessels for a period of 1-1½ hours a progressive and severe asphyxia with a final arterial pH of 6.90 was achieved. Varying the mean arterial blood pressure in the fetuses by blood withdrawal or infusion in this state CBF was measured at different perfusion pressures (mean arterial blood pressure (MABP) minus central venous pressure (CVP)). A passive flow/pressure relationship—loss of autoregulation—was found with hyperemia reaching CBF values up to 5 times normal at normal MABP of about 60 to 70 mmHg and severe ischemia reaching CBF values close to zero in large cortical areas at MABP of 30 mmHg. CVP remained essentially unchanged at 10-15 mmHg. The severe and prolonged asphyxia rendered the blood-brain barrier leaky to the albumin tracer Evans blue. In four other fetuses umbilical cord clamping was omitted. However only in one of these cases was acidosis completely avoided and CBF autoregulation maintained. The three other fetuses were acidotic at the end of the surgical procedure and had impaired autoregulation.

KEY WORDS Asphyxia: autoregulation blood brain barrier cerebral ischemia fetal circulation hypotension hypoxia regional cerebral blood flow

Courville (5) has pointed to *cerebral ischemia* as a possible complicating factor contributing to brain damage in the distressed newborn. Not only is the arterial blood poorly oxygenated but perhaps even the volume of this blood supply may be severely reduced. The concept has gained support from the findings of Schwartz (28) that nearly all infants dying during the first days after birth have brain tissue infarcts located to border zones between arterial territories. Direct evidence of brain ischemia in prolonged and severe asphyxia was obtained by Reivich et al (27) studying fetal monkeys.

The immediate response to fetal asphyxia is *hypertension*. But as shown by Dawes et al (6) *hypotension* eventually develops. Thus we believe may be the mechanism leading to

cerebral ischemia in asphyxia if normal autoregulation of cerebral blood flow is lacking. The aim of the present study therefore has been to establish if cerebral blood flow is constant or not in the face of variations of arterial blood pressure in asphyxiated sheep fetuses.

MATERIALS AND METHODS

The study involved 17 near term pregnant sheep. The sheep were anaesthetized by intrathecal lumbar injection of 2-4 ml 2% lidocaine. The abdomen was opened by a transverse incision in the right iliac region. One upper and one lower extremity were successively delivered through small incisions in the uterus. Care was taken not to interfere with placental circulation and to minimize the exposure of uterus and fetal limbs to the exterior environment. A polyethylene catheter was introduced into a brachial artery the tip being placed in the brachio-cephalic trunk supplying upper extremities and the head. Catheters were

Table 2 CBF measurements in severely asphyxiated fetuses (see Figs 1 and 2 for a description of the main findings)

	pH	Paco ₂ (mmHg)	Paco ₂ (mmHg)	MABP (mmHg)	CVP (mmHg)	rCBF (ml/g/min)	pH	Paco ₂ (mmHg)	Paco ₂ (mmHg)	MABP (mmHg)	CVP (mmHg)	rCBF (ml/g/min)
<i>Group A Decrease in arterial blood pressure</i>												
II	6.89	58	17	60	10	co 5.96 pv 14.2 bs 13.8 ce 6.60	6.83	65	15	50	10	co 3.84 pv 4.11 bs 1.84 ce 0.59
JJ	6.9	50	7	80	10	co 4.67 pv 10.4 bs 14.2 ce 8.18	6.97	54	2	40	10	co 1.10 pv 3.27 bs 4.79 ce 2.52
KK	6.78	80	15	55	10	co 7.11 pv 4.09 bs 4.03 ce 7.87	6.79	80	15	30	10	co 0.05 pv 0.08 bs 0.08 ce 0.03
LL	6.99	68	18	75	10	co 3.58 pv 8.46 bs 8.39 ce 4.67	6.99	71	18	55	10	co 1.32 pv 3.17 bs 4.38 ce 2.70
Mean	6.90	64	17	68	10	co 4.08 pv 9.31 bs 10.1 ce 5.58	6.88	68	17	44	10	co 1.58 pv 3.15 bs 3.15 ce 1.48
<i>Group B Increase in arterial blood pressure</i>												
BB	6.94	70	16	45	15	co 1.79 pv - bs 3.50 ce 2.87	7.17	39	31	85	15	co 0.864 pv - bs 1.68 ce 2.09
DD	6.83	59	17	45	10	co 0.95 pv 2.48 bs 3.13 ce 1.71	6.75	48	28	75	10	co 1.75 pv 3.87 bs 4.98 ce 2.00
FF	6.84	54	13	50	15	co 1.98 pv 4.00 bs 4.70 ce 2.48	6.85	54	24	90	15	co 2.10 pv 4.31 bs 4.83 ce 2.24
HH	6.9	47	8	50	10	co 1.40 pv 4.20 bs 4.76 ce 2.7	6.97	47	78	60	10	co 1.40 pv 2.79 bs 3.0 ce 3.78
Mean	6.88	56	17	47	13	co 1.54 pv 3.56 bs 3.90 ce 2.83	6.84	48	77	75	13	co 1.75 pv 3.64 bs 4.33 ce 2.51

BB excluded as cuff was released co Cortex pv Periventricular region bs Brain stem ce Cerebellum MABP Mean arterial blood pressure CVP Central venous pressure rCBF Regional cerebral blood flow

ments (JJ and MM). Approximately 10^4 spheres with 4×10^4 counts were injected during continuous agitation from a special vial into the inferior vena cava. The concentration of microspheres in the blood on its way to the brain was measured by counting 5 ml blood withdrawn continuously during a period of 2 min from the brachiocephalic trunk. The blood sample was counted in a Chicago⁹ well type nuclear gamma spectrometer. The

injection of the second batch of microspheres (the order randomly chosen) took place approximately 10 min later. rCBF was calculated from the formula

$$CBF = \frac{F \cdot \text{tissue}}{I}$$

(F Sampling rate of arterial blood 1/min and I counting rates of cerebral tissue and of arterial blood)

Table 1 Control state after termination of surgical procedure

Sheep	Weight (kg)	pH	P _a CO ₂ (mmHg)	P _a O ₂ (mmHg)	MABP (mmHg)	CVP (mmHg)
BB	1.1	7.35	35	19	55	12
DD	4.5	7.24	34	16	75	15
FI	4.1	7.35	35	17	80	15
HH	4.0	7.38	27	21	50	10
II	4.2	7.31	40	14	75	10
JJ	4.6	7.36	36	14	75	10
KK	4.2	7.22	44	14	65	10
LL	3.7	7.31	34	22	60	10
JJJ	3.0	7.27	36	18	58	10
LLL	3.0	7.19	38	17	76	10
MMM	3.0	7.33	30	19	55	10
PPP	2.8	7.25	24	24	60	10
Mean	3.7	7.30	34	18	65	11
S.D.	0.66	0.06	5.4	3.3	10	2.0
S.E.	0.19	0.02	1.6	0.9	3.0	0.6

In these acute preparations fetal hypocapnia was a general finding probably due to maternal hyperventilation. Several fetuses were hypoxic and/or slightly acidotic.

also placed in the inferior vena cava (from femoral vein) and in a femoral artery. Both were connected to pressure transducers (Statham®) and in this way mean arterial blood pressure (MABP) and central venous pressure (CVP) were recorded continuously. pH was monitored at least once every 10 min and P_aO₂ and P_aCO₂ using Radiometer® electrodes at more irregular intervals in arterial blood samples from the brachio-cephalic trunk. The values were corrected for temperature. Rectal temperature was measured in the ewes. All were normothermic. After positioning the catheters a small inflatable cuff was placed around the umbilical cord in eight fetuses. The surgical procedure was then terminated by suturing the abdomen.

State after termination of the surgical procedure

After completion of the surgical procedure the state of the 12 fetuses was determined (Table 1). These acute preparations were all hypocapnic and several slightly acidotic and hypoxic. (Normal values in chronic preparations: pH 7.445±0.006, P_aCO₂ 21.4±1.0, P_aCO₂ 53.8±0.9 (23)) probably due to maternal hyperventilation and stress during the operation. These deviations were considered to be of minor importance in view of the fact that a similar state is typical in relation to normal birth.

Induction of severe and prolonged asphyxia

In eight experiments the non-extensorized fetus was gradually asphyxiated by inflating the umbilical cuff to a pressure near the mean arterial blood pressure. The fetuses reacted initially by hypertension with a mean increase of 19 mmHg. The maximal MABP was reached after 30 min and in most cases maintained during another 30 min. Then the MABP gradually fell. When pH had reached values of about 6.90 the MABP had returned to the initial levels of 60–75 mmHg. In four of these cases (group A, Table 2) the first CBF measurement took place at this point 60–90 min after starting partial cord constriction. The measurement was repeated about 10 min later after

the MABP had been lowered to a mean of 44 mmHg by withdrawal of 40–50 ml blood.

In 4 other fetuses which were asphyxiated for a similar period of time and to a similar degree (group B, Table 2) the blood withdrawal was carried out immediately before the first CBF measurement which took place at a MABP of 47 mmHg (mean value). The second measurement was carried out about 10 min later after re-infusion of blood to a mean MABP of 75 mmHg.

Fetuses without cord constriction

In the remaining 4 fetuses (Table 3) no attempt was made to induce asphyxia. After the surgical procedure had been terminated one fetus had normal arterial pH (MMM) the other three were slightly acidotic (Table 1). At the time of the first CBF measurement (a few minutes later) all except MMM were moderately hypoxic. CBF was measured at different MABPs as described above. However it proved to be considerably more difficult to achieve significant blood pressure changes by bleeding or re-infusion in these less asphyxiated fetuses. 90–110 ml of blood had to be withdrawn or infused to obtain appreciable pressure changes.

In two experiments three types of isotope labelled microspheres were used allowing three CBF measurements at different MABPs (see below).

Determination of rCBF

The inert microsphere technique as described by Hales (14) was used. This newly developed method is now regarded as the most accurate method in experimental animals (16). The microspheres were made of plastic material having a specific gravity slightly greater than that of whole blood (1.1–1.6 compared with approximately 1.05) with a diameter of 15 µm. In most cases two batches were used: one labelled with ⁸⁶Sr, one with ¹⁴¹Ce (3M Company, St Paul) allowing two CBF measurements in each experiment. In two experiments also ⁵¹Cr marked microspheres were used allowing three CBF measure-

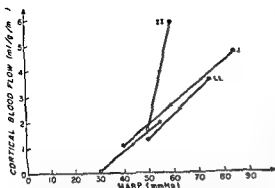


Fig 1 Cortical blood flow (average of 8 determinations) measured at normal MABP and subsequently at decreased MABP in each of 4 sheep fetuses. Very high flows are recorded in severe normotensive asphyxia but CBF drops proportionally or even more as MABP decreases. Autoregulation is abolished (Table 2A).

very high up to 5.9 ml/g/min in cortex and 14 ml/g/min in the periventricular regions and brain stem. These values are 4–6 times normal (23) and indicate an extreme decrease in vascular resistance. Vasodilatation. After induction of moderate or severe hypotension the flows fall dramatically. CBF is pressure passive. In other words normal autoregulation is abolished.

Table 2B illustrates the CBF in severe hypotensive asphyxia followed by induction of normotension after re-infusion of blood. It is seen that restoration of normotension fails to raise CBF appreciably in these severely asphyxiated fetuses. This phenomenon may be termed false autoregulation and is also seen in other pathological states of the brain (3). It is probably due to increased oedema formation and raised intracranial pressure.

Table 3 shows the CBF at different MABPs in the fetuses without cord constriction. In the case of the best preserved fetus (MMM) flow is essentially unchanged at decreased and increased MABP. Autoregulation is preserved. PPP which was still acidotic had completely lost autoregulation. The 2 other fetuses were less affected.

The comparative distribution of CBF in different regions of the brain (periventricular re-

gion, cerebellum and brain stem) show a consistent pattern. The regions near the neuraxis (periventricular region and brain stem) have by far the highest flows. About 2.5 times that of the cortex (Tables 1, 2 and 3).

In vivo injection of Evans blue at the termination of prolonged and severe asphyxia resulted in a marked coloration throughout the basal ganglia and especially the cortex indicating a breakdown of the blood-brain barrier to albumin.

In the control twins Evans blue was visible only in major veins.

DISCUSSION

The present demonstration of extremely high CBF in normotensive severe fetal asphyxia is expected. As mentioned in the introduction increased CBF is the usual consequence of asphyxia as both hypoxia and hypercapnia with increased extracellular acidosis are important vasodilator stimuli (9, 18, 19). Even CBF prior to cord constriction is in accordance with Makowski et al. much higher than in normal adults probably due to the fact that fetal blood is much less oxygenated (23).

If autoregulation is preserved hypotension will not produce any change in CBF or in the concentrations of energy rich phosphates in the brain provided the hypotension does not become excessive (1, 2, 18, 20, 26). We found that a moderate decrease in the mean arterial blood pressure leads to a proportional (or even more pronounced) decrease in the very high cerebral blood flow of the severely asphyxiated fetus. This must constitute a breakdown of a compensatory mechanism leading to an increase in tissue hypoxia and acidosis.

The failure of seeing a flow increase when the reversing order of the experiment (group B, Table 2) is surprising. Since impaired autoregulation was seen even in minimally asphyxiated control studies (Table 3, Fig. 3) the constancy of flow in group B might be related to the CBF constancy despite variation in perfusion pressure seen in the most severely dam-

Table 3 *Fetuses without cord constriction (see Fig 3 for a description of the main findings)*

	pH	Paco ₂ (mmHg)	Pao ₂ (mmHg)	MABP (mmHg)	CVP (mmHg)	rCBF (ml/g/min)
<i>First CBF measurement</i>						
JJJ	7.33	32	14	54	10	co 1.66 pv 2.06 bs 2.44 ce 2.21
LLL	7.34	28	15	75	10	co 1.69 pv 2.50 bs 3.46 ce 2.93
MMM	7.33	30	19	55	10	co 0.98 pv 1.29 bs 1.66 ce 1.63
PPP	7.28	38	17	60	10	co 1.45 pv 2.57 bs 3.00 ce 2.22
<i>Second CBF measurement</i>						
JJJ	7.35	30	17	45	10	co 1.11 pv 1.42 bs 1.32 ce 1.52
LLL	7.32	30	17	60	10	co 0.96 pv 1.49 bs 2.28 ce 1.97
MMM	7.34	28	22	45	10	co 1.08 pv 1.42 bs 1.90 ce 1.88
PPP	7.27	39	21	48	10	co 0.56 pv 0.89 bs 1.16 ce 1.01
<i>Third CBF measurement</i>						
JJJ	7.31	29	17	55	10	co 1.67 pv 2.44 bs 2.33 ce 2.44
MMM	7.33	28	23	64	10	co 1.21 pv 2.24 bs 2.56 ce 2.36

Blood-brain barrier studies

After the last rCBF measurement 12 ml of a 2% aqueous solution of the albumin tracer Evans blue was injected slowly intravenously (2 min injection time). The tracer was allowed to circulate for 15 min. In two cases the presence of a twin fetus made it possible to perform a control study on the non asphyxiated fetus.

After termination of the experiment the ewe and the fetus were killed by intravenous injection of barbiturate followed by KCl. The fetal brain was removed and 1–2 g samples were taken for counting from the following regions: 4 samples from the left hemisphere cortex; 4 from

the right hemisphere cortex; 1 from the periventricular area (thalamus, hypothalamus, basal ganglia and adjacent white matter); 1 from the brain stem and 1 from cerebellum. The tubes of the spectrometer were filled to a height of less than 2 cm and counted in the same manner as the blood samples (14). At autopsy the correct placement of catheters was ascertained.

RESULTS

Fig 1 and Table 2A show the regional CBF in severe normotensive asphyxia. The flows are

- mature infants with low serum bilirubin concentrations *Pediatrics* 45 906 1970
- 13 Haggendal E & Johansson B On the pathophysiology of increased cerebrovascular permeability in acute arterial hypertension in cats *Acta Neurol Scand* 48 65 1972
 - 14 Hales J R ■ Radioactive microsphere techniques for studies of the circulation *Clin Exp Pharmacol Physiol Suppl* 1 31 1974
 - 15 Hambleton G & Wigglesworth J S Onset of intraventricular haemorrhage in the preterm infant *Arch Dis Child* 59 651 1976
 - 16 Heistad D O Marcus M L & Mueller S Measurement of cerebral blood flow with microspheres *Arch Neurol* 34 657 1977
 - 17 Johansson B Blood-brain barrier dysfunction in acute arterial hypertension after papaverine induced vasodilatation *Acta Neurol Scand* 50 573 1974
 - 18 Johansson H & Siesjö B K Cerebral blood flow and oxygen consumption in the rat in hypoxic hypoxia *Acta Physiol Scand* 93 769 1975
 - 19 Lassen N A Brain extracellular pH The main factor controlling cerebral blood flow *Scand J Clin Lab Invest* 22 747 1968
 - 20 Lassen N A Discussion In *Cerebral Blood Flow Regulation Proc Fifth Intern Symp Roma-Siena 1971 European Neurol* 8 279 1972
 - 21 Leech R W & Alvord E C Morphological variations in periventricular leukomalacia *Am J Pathol* 74 591 1974
 - 22 Lou H C Tweed W A Johnson G Jones M & Lassen N A Break-down of the blood-brain barrier in kernicterus *Lancet* 1 1067 1977
 - 23 Makowski E L Schneider J M Tsoulos N G Colwill J E Battaglia F C & Meschia G Cerebral blood flow oxygen consumption and glucose utilization of fetal lambs in utero *Am J Obstet Gynecol* 114 797 1972
 - 24 Mossakowski M J Long D M Myers R E Rodriguez de Curet H & Klatzo I Early histochemical changes in perinatal asphyxia *J Neuropath Exp Neurol* 27 500 1968
 - 25 Myers R E Four patterns of perinatal brain damage and their conditions of occurrence in primates In B S Meldrum & D C Masden (eds) *Advances in Neurology* Raven Press 10 23 1975
 - 26 Purves J M & James I M Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb *Circ Res* 25 651 1969
 - 27 Reivich M Brann A W Shapiro H M & Myers R E Regional cerebral blood flow during prolonged partial asphyxia In J S Meyer (ed) *Research on Cerebral Blood Flow* Ch C Thomas Springfield Ill 1973 p 217
 - 28 Schwartz P Birth injuries In J Minkler *Pathology of the Nervous System* McGraw Hill N Y 1971 p 1779

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aged cases of stroke and brain trauma (10-11) (false autoregulation) and it is speculated that the local tissue pressure rises during arterial pressure increase to offset an increase in perfusion pressure.

The finding that even minimal acidosis impairs autoregulation in the fetus is also unexpected. The explanation may well be that the arterioles already are somewhat dilated in the normal fetal state due to arterial hypoxia in comparison to the postnatal state.

Our experimental demonstration of critically low cerebral perfusion in hypotensive perinatal distress would explain why hypoxic tissue damage is mainly seen in watershed regions between vascular territories in the brain (7, 8, 21, 25).

The fact that autoregulation is lacking in the hypoxic fetus will render the capillary walls more directly exposed to an eventual increase in arterial blood pressure and in the germinal layer of the very immature brain the rich capillary network is not supported by rigid glial structures (15). This may well explain why germinal layer capillary bleeding is a frequent complication to cerebral hypoxia in the premature infant.

Blood-brain barrier damage

The present study also demonstrated that prolonged severe asphyxia leads to pronounced damage to the blood-brain barrier in particular in the cortex and to a lesser extent in the basal ganglia. Mossakowski et al have previously reported rare foci of blue coloration following Evans blue injection after short lasting total asphyxia in monkeys (24). The dye was seen primarily inside the neurones.

It is most likely that the breakdown of the blood-brain barrier is not caused directly by the tissue hypoxia but to its indirect effect. Maximal vasodilatation in combination with moderate arterial hypertension. The breakdown of the blood-brain barrier and oedema may further aggravate circulatory disturbances in severe and prolonged asphyxia (13, 17).

The passage of albumin from plasma to neurones will enhance transport of albumin bound bilirubin to the neurones and this offers an explanation of the increased susceptibility of small asphyxiated infants to kernicterus (12, 22).

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REFERENCES

1. Brierley J B, Brown A W, Excell B J & Meldrum B S. Brain damage in the rhesus monkey resulting from profound arterial hypotension. *Brain Res* 13: 68, 1969.
2. Brierley J B & Excell B J. The effects of profound systemic hypotension upon the brain of Macacus rhesus. *J Physiol (Lond)* 175: 50P, 1964.
3. Bruce D A, Langfitt T W, Miller J D, Schutz H, Vapalakki M, Stanek A & Goldberg H I. Regional cerebral blood flow, intracranial pressure and brain metabolism in comatose patients. *J Neurosurg* 38: 131, 1973.
4. Chu J, Clements J A, Cotton E A, Klaus M H, Sweet A Y & Tolley W H. Neonatal pulmonary ischemia. Part I. Clinical and physiological studies. *Pediatrics* 40: 709, 1967.
5. Courville C M. Antenatal and perinatal circulatory disorders as a cause of cerebral damage in early life. *J Neuropathol Exp Neurol* 18: 115, 1959.
6. Dawes C S. *Fetal and Neonatal Physiology*. Year Book Medical Publishers Inc, Chicago, 1968, p. 143.
7. De Reuck J, Chattha S A & Richardson E P. Pathogenesis and evolution of perinatal leukomalacia in infancy. *Arch Neurol* 27: 229, 1972.
8. van der Eecken H. *Anastomoses between the Leptomeningeal Arteries of the Brain*. Ch C Thomas, Springfield, 1959.
9. Elliott K A C & Jesper H H. Physiological salt solutions for brain surgery. Studies of local pH and pial vessels reactions to buffered and unbuffered isotonic solutions. *J Neurosurg* 6: 140, 1949.
10. Enevoldsen E & Jensen S T. Autoregulation and CO₂ reactivity of cerebral blood flow in patients with severe head injury. *J Neurosurg* in press.
11. Fieschi C, Bathusini N, Beduschi A, Boselli L & Rossanda M. Regional cerebral blood flow and intraventricular pressure in acute head injuries. *J Neurol Neurosurg Psychiatry* 37: 1378-1388, 1974.
12. Gartner L M, Snyder H N, Chabon R S & Bernstein J. Kernicterus. High incidence in pre-

SERUM RETINOL BINDING PROTEIN AND VITAMIN A LEVELS IN MALNOURISHED CHILDREN

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ABSTRACT Reddy V Mohanram M and Raghuramulu N (National Institute of Nutrition Hyderabad India) Serum retinol binding protein and vitamin A levels in malnourished children *Acta Paediatr Scand* 68 1979—Serum levels of vitamin A and retinol binding protein (RBP) were measured in children with vitamin A deficiency in children with protein energy malnutrition (PEM) and in normal children before and after administration of 100 000 IU of water miscible vitamin A. Serum vitamin A and RBP levels were significantly low in children with vitamin A deficiency and in children with severe PEM whereas the values in milder grades of PEM were similar to those of normal subjects. In severely malnourished children with corneal lesions serum vitamin A concentration was reduced to a much greater extent than the level of serum RBP. Administration of vitamin A resulted in a significant increase in serum levels of both the components within 4 hours in all the 3 groups of children. The increase in RBP concentration observed in children with PEM was similar to that in vitamin A deficient children. These results indicate that in malnourished children particularly in those who are at risk of developing keratomalacia vitamin A is the main limiting factor. It is therefore recommended that children with PEM should be treated with vitamin A in addition to dietary protein and calories.

KEY WORDS vitamin A malnutrition retinol binding protein

Protein-energy malnutrition (PEM) and vitamin A deficiency are the two most frequently encountered deficiency diseases among children in many developing countries. Ocular signs of vitamin A deficiency are seen in 30-40% of the children suffering from kwashiorkor (1). Levels of serum vitamin A are low in children with PEM whether or not they exhibit clinical signs of vitamin A deficiency (10). Recent studies suggest that retinol transport system is defective in this condition (13 15 16). Smith et al (13) reported that in children with kwashiorkor low serum vitamin A levels were associated with decreased concentration of retinol binding protein (RBP) and pre albumin. Treatment with calories and protein but without supplemental vitamin A resulted in a significant increase in the concentration of all the three components. It was therefore suggested that low levels of serum

vitamin A may reflect a defective hepatic production of the carrier protein rather than vitamin A deficiency per se. However some children with kwashiorkor have associated vitamin A deficiency and experiments in rats have shown that vitamin A status *per se* can modify RBP metabolism (6 7 9). Studies were therefore undertaken to examine the effect of vitamin A deficiency on the vitamin A transport system in malnourished children. Serum RBP and vitamin A levels were measured in children with vitamin A deficiency and in children with PEM before and after administration of vitamin A.

SUBJECTS AND METHODS

Ninety three children aged between 1-8 years were investigated. They were classified into 4 groups based on their nutritional status.

similar in children with vitamin A deficiency and in children with PEM (Fig 1)

DISCUSSION

In the present study serum levels of vitamin A and RBP were found to be low in children with vitamin A deficiency (group III) and administration of vitamin A resulted in a significant increase in the concentration of both the components within 4 hours. These observations are in line with those reported in rats (6, 7, 9) and suggest that the release of RBP from the liver depends on the availability of vitamin A. Since these children did not have associated PEM of severe form, synthesis of RBP may not be limited. Following the massive dose of vitamin A, the rise in serum vitamin A was much more marked than the rise in RBP, suggesting that a part of the circulating vitamin was still in the ester form at 24 hours.

In undernourished children, serum levels of vitamin A and RBP were not different from those of normal subjects, indicating that the vitamin transport system was not affected by mild degrees of PEM. Whereas in children with severe PEM, serum levels of both the components were significantly reduced—an observation similar to that reported by other workers (13, 15, 16). The regulation of serum vitamin A concentration in PEM seems to be influenced by protein nutrition as well as vitamin A status of the children. Studies in Egyptian children with kwashiorkor have shown that treatment with protein and calories, but without vitamin A, resulted in increased levels of serum vitamin A and RBP after 2–4 weeks (15). These observations suggest that protein-calorie deficiency may influence vitamin A metabolism by interfering with hepatic synthesis of RBP. In the studies reported here, administration of vitamin A to children with kwashiorkor resulted in a rapid and significant increase in serum vitamin A as well as serum RBP within 4 hours, before any change in protein nutrition could occur. These results are similar to those observed in vitamin A defi-

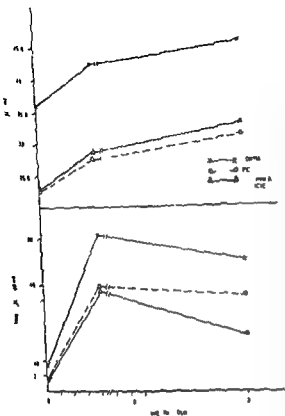


Fig 1 Effect of vitamin A administration on serum levels of vitamin A and RBP

and 39.9 $\mu\text{g}/\text{ml}$ respectively. The values observed in undernourished children (group II) were not different from those of normal children. Serum levels of vitamin A and RBP were significantly lower in vitamin A deficient children (group III) and in children with PEM (group IV) as compared to normal subjects (group I). The mean concentrations were significantly lower in malnourished children with corneal lesions than in those without ocular signs (Table 1).

Following the administration of vitamin A, there was a significant increase in serum vitamin A and RBP levels both at 4 hours and 24 hours in all the 3 groups of children studied. However, the percentage increase in serum RBP concentration was greater in malnourished children than in normals (Table 2). Response to vitamin A administration was

Table 1 Serum vitamin A and RBP levels in children

Values are mean \pm SE

Groups	No of subjects	Serum albumin (g/100 ml)	Serum vitamin A (μ g/100 ml)	Serum (RBP μ g/ml)
I Normal	26	3.2 \pm 0.02	38.5 \pm 2.77	39.9 \pm 2.26
II Undernourished	12	2.8 \pm 0.08	31.4 \pm 4.15	34.5 \pm 3.46
III Vitamin A deficiency	13	2.7 \pm 0.19	17.7 \pm 2.36	22.8 \pm 1.77
IV Severe PEM				
(a) Without vitamin A deficiency signs	14	1.70 \pm 0.16	19.2 \pm 2.31	27.1 \pm 3.86
(b) With conjunctival lesions	11	1.30 \pm 0.19	17.3 \pm 3.55	27.2 \pm 3.61
(c) With corneal lesions	17	1.40 \pm 0.12*	6.1 \pm 0.85	19.6 \pm 1.07

* $p < 0.001$ as compared to group I

Group I 26 apparently normal children aged 2-8 years. Their weights were above 80% of the local standard (3).

Group II 12 undernourished children aged 2-8 years. Their weights were below 80% of the standard but they had no clinical signs of PEM.

Group III 13 children aged 3-8 years who had ocular signs of vitamin A deficiency like conjunctival xerosis and/or bitot spots. Their weights ranged from 70-90% of the standard and they had no clinical signs of PEM.

Group IV 42 children with severe PEM aged 1-5 years. Most of them were of marasmic kwashiorkor type showing oedema as well as emaciation. 14 children had no clinical signs of vitamin A deficiency while 11 children had conjunctival xerosis and/or bitot spots (X₁, A and B) and 17 children had serious corneal lesions of X₂ and X₃ type of WHO nomenclature (17).

Fasting blood samples were collected from all the children initially. Ten normal children of group I, 13 vitamin A deficient children of group III and 20 children with PEM in group IV including all the 17 with corneal lesions were administered a single dose of 100 000 I.U. (30 000 μ g) of water miscible vitamin A palmitate (Aquesol) intramuscularly and blood samples were collected at 4 hours and 24 hours after the dose. Serum vitamin A was determined

in duplicate by a micro fluorometric method (11). The vitamin A values obtained by this method for malnourished children were in close agreement with the standard macro procedures (12). Serum RBP was assayed by immunodiffusion technique (5) the standard human RBP and human anti RBP were obtained from Behringwerke West Germany. Serum albumin was determined by the dye method (2).

RESULTS

Undernourished children (group II) and vitamin A deficient children (group III) had slightly lower levels of serum albumin as compared to those of normal subjects. Whereas the mean concentration of serum albumin was significantly decreased in children with severe PEM. In normal children the mean levels of serum vitamin A and RBP were 38.5 μ g/100 ml

Table 2 Effect vitamin A administration* on serum vitamin A and RBP levels

Values are mean \pm SE

Group	No of subjects	Vitamin A μ g/100 ml			RBP μ g/ml		
		0 hr	4 hr	24 hr	0 hr	4 hr	24 hr
I Normal	10	33.8 \pm 4.08	205.0 \pm 62.75**	170.8 \pm 67.96**	36.5 \pm 3.38	42.2 \pm 3.70**	45.6 \pm 4.10**
II Vitamin A deficiency	13	17.8 \pm 6.89	131.6 \pm 98.27***	76.2 \pm 61.27**	23.0 \pm 6.38	29.1 \pm 8.30	33.3 \pm 10.12
III Severe PEM	20	15.9 \pm 2.65	139.6 \pm 35.44**	125.5 \pm 24.13***	22.8 \pm 2.24	27.7 \pm 2.35	31.4 \pm 2.78*

100 000 I.U. vitamin A (Aquesol)

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.0001$ as compared to 0 hr

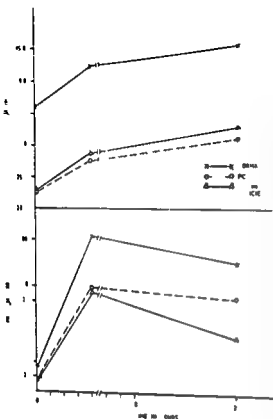


Fig 1 Effect of vitamin A administration on serum levels of vitamin A and RBP

and 39.9 $\mu\text{g/ml}$ respectively. The values observed in undernourished children (group II) were not different from those of normal children. Serum levels of vitamin A and RBP were significantly lower in vitamin A deficient children (group III) and in children with PEM (group IV) as compared to normal subjects (group I). The mean concentrations were significantly lower in malnourished children with corneal lesions than in those without ocular signs (Table 1).

Following the administration of vitamin A there was a significant increase in serum vitamin A and RBP levels both at 4 hours and 24 hours in all the 3 groups of children studied. However the percentage increase in serum RBP concentration was greater in malnourished children than in normals (Table 2). Response to vitamin A administration was

similar in children with vitamin A deficiency and in children with PEM (Fig 1).

DISCUSSION

In the present study serum levels of vitamin A and RBP were found to be low in children with vitamin A deficiency (group III) and administration of vitamin A resulted in a significant increase in the concentration of both the components within 4 hours. These observations are in line with those reported in rats (6, 7, 9) and suggest that the release of RBP from the liver depends on the availability of vitamin A. Since these children did not have associated PEM of severe form, synthesis of RBP may not be limited. Following the massive dose of vitamin A, the rise in serum vitamin A was much more marked than the rise in RBP suggesting that a part of the circulating vitamin was still in the ester form at 24 hours.

In undernourished children serum levels of vitamin A and RBP were not different from those of normal subjects indicating that the vitamin transport system was not affected by mild degrees of PEM. Whereas in children with severe PEM serum levels of both the components were significantly reduced—an observation similar to that reported by other workers (13, 15, 16). The regulation of serum vitamin A concentration in PEM seems to be influenced by protein nutrition as well as vitamin A status of the children. Studies in Egyptian children with kwashiorkor have shown that treatment with protein and calories but without vitamin A resulted in increased levels of serum vitamin A and RBP after 2–4 weeks (15). These observations suggest that protein-calorie deficiency may influence vitamin A metabolism by interfering with hepatic synthesis of RBP. In the studies reported here administration of vitamin A to children with kwashiorkor resulted in a rapid and significant increase in serum vitamin A as well as serum RBP within 4 hours before any change in protein nutrition could occur. These results are similar to those observed in vitamin A defi-

cient states and suggest that in PEM vitamin A is a limiting factor. While protein deficiency may cause impaired synthesis of RBP, vitamin A deficiency interferes with its release from the liver. Thus deficiencies of both the nutrients seem to contribute to lowering of serum vitamin A concentration in children with PEM.

Under physiological conditions, retinol and RBP are present in serum in equimolar ratio and after retinol is taken up by the target tissues, free RBP is metabolised by the kidney (8). In normal subjects, only 15–20% of the total RBP is in the apo form (14). In severely malnourished children with corneal lesions studied here, although serum vitamin A concentration was considerably lowered, the RBP concentration did not fall to the same extent. The ratio of RBP:retinol was therefore raised, indicating that vitamin A levels were reduced in spite of carrier protein being available. It is possible that in severe vitamin A deficiency, uptake of retinol by the depleted tissues may be very rapid, resulting in a higher concentration of apo RBP in serum. However, this can be answered only by determining the levels of apo and holo RBP separately.

Although the authors have not commented on it, similar results have been obtained in children with PEM studied by Smith et al (13, 15). A careful look at their data reveals that in children with marasmic kwashiorkor who had poor hepatic stores of vitamin A, the concentration of serum vitamin A was reduced to a much greater extent than was the level of serum RBP, suggesting that the ratio of holo to apo RBP was altered.

These findings are of considerable significance since they indicate that in malnourished children, particularly in those who are at risk of developing keratomalacia, vitamin A is the main limiting factor. In Egyptian and central American children with PEM, although serum vitamin A levels were low, none of them had serious corneal involvement like the Indian children studied here. This difference can be explained on the basis of vitamin A status of

the children. PEM alone can lead to a moderate reduction in the serum concentration of both RBP and vitamin A, but when there is associated vitamin A deficiency, serum vitamin A concentration decreases further, increasing the risk of keratomalacia. Thus, the role of vitamin A seems to be more critical than that of protein in the development of keratomalacia and blindness.

Children with PEM should therefore be treated with vitamin A in addition to dietary protein and calories. This is of utmost importance in case of corneal involvement. Vitamin A deficiency can be corrected rapidly, while improvement in protein nutrition is a slow process.

A single injection of 100 000 I.U. (30 000 µg) of water-miscible vitamin A will not only produce an increase in serum vitamin A concentration but will also result in rapid clinical improvement. We have observed that early corneal xerosis clears up completely and vision is restored to normal within a few days after the dose, but in more advanced cases of keratomalacia, scar formation is inevitable. Oral administration of vitamin A has also been found to be effective, but parenteral administration is preferable since a majority of children with PEM have associated gastroenteritis.

Malnourished children admitted to the hospital were given vitamin A in addition to dietary protein and calories. Though this is the best method of treatment, some children with PEM and corneal xerosis were treated as outpatients because their parents refused admission. It was observed that administration of vitamin A alone resulted in complete reversal of corneal xerosis. The vision could be saved though there was no improvement in the general condition. These observations confirm the above hypothesis and have a bearing on the treatment of this condition.

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REFERENCES

- Chandra H, Venkatachalam P S, Belavady B, Reddy V & Gopalan C. Some observations on vitamin A deficiency in Indian children. *Indian J Child Health* 9: 589 1960.
- Doumas B T, Watson W A & Biggs H G. Al human standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 31: 87 1971.
- Indian Council of Medical Research (ICMR). Growth and physical development of Indian infants and children. *Tech Rep Series* No 18 1972.
- Ingenbleek Y, Schneek H G V, DeNayer P & Devisscher M. The role of retinol binding protein in protein-calorie malnutrition. *Metabolism* 24: 633 1975.
- Mancini G, Carbonara A O & Heremans J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Int J Immunochem* 2: 235 1965.
- Mukhlal H & Glover J. Effect of dietary deficiencies of protein and retinol on the plasma retinol binding protein in the rat. *Br J Nutr* 32: 549 1974.
- Muto Y, Smith J E, Milch P O & Goodman D S. Regulation of retinol binding protein metabolism by vitamin A status in the rat. *J Biol Chem* 247: 2541 1972.
- Peterson P A, Nilsson S F, Östberg L, Rask L & Vahlquist A. Aspects of the metabolism of retinol binding protein and retinol. *Vitam Horm* 32: 181 1974.
- Peterson P A, Rask L, Östberg L, Andersson L, Kemwendo F & Pertoft H. Studies on the transport and cellular distribution of vitamin A in normal and vitamin A deficient rats with special reference to the vitamin binding plasma protein. *J Biol Chem* 248: 4009 1973.
- Reddy V & Sankanta S G. Serum vitamin A in kwashiorkor. *Am J Clin Nutr* 18: 105 1966.
- Selvaraj R J & Susheela T. Estimation of serum vitamin A by a microfluorometric procedure. *Clin Chim Acta* 27: 165 1970.
- Sivakumar M. Evaluation of a microspectrofluorometric method for serum vitamin A. *Clin Chim Acta* 79: 189 1977.
- Smith F R, Goodman D S, Arroyave E & Viten F. Serum vitamin A, retinol binding protein and prealbumin concentrations in protein-calorie malnutrition. II. Treatment including supplemental vitamin A. *Am J Clin Nutr* 26: 987 1973.
- Smith F R, Raz A & Goodman D S. Radioimmunoassay of human plasma retinol binding protein. *J Clin Invest* 49: 1754 1970.
- Smith F R, Goodman D S, Zaklana M S, Gabr M K, Maraghy E & Patwardhan V N. Serum vitamin A, retinol binding protein and prealbumin concentration in protein calorie malnutrition. I. A functional defect in hepatic retinol release. *Am J Clin Nutr* 26: 973 1973.
- Venkataswamy G, Glover J, Cobby M & Pine A. Retinol binding protein in serum of xerophthalmic malnourished children before and after treatment at a nutrition centre. *Am J Clin Nutr* 30: 1968 1977.
- Vitamin A deficiency and xerophthalmia. WHO Tech Rep Series No 390 1976.

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FINGERPRINTS IN CONGENITAL RUBELLA FOLLOWING MATERNAL GAMMA GLOBULIN

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ABSTRACT Ross L. J. (Department of Pediatrics, New York University Medical Center, New York, U.S.A.): Fingerprints in congenital rubella following maternal gamma globulin. *Acta Paediatr Scand* 68 71 1979.—The fingertip skin ridge patterns of ten children with the congenital rubella syndrome whose mothers had received gamma globulin following exposure to German measles during pregnancy were compared with those of 29 patients whose mothers had not received gamma globulin and with those of 162 control children. Statistical evaluation of the pattern profiles by the Mann-Whitney U test showed that the dermatoglyphics of the two groups of patients differed significantly from each other. Each group also differed from the control children. There was a significant ($p < 0.001$) increase in whorls in patients whose mothers had not had gamma globulin but an increase ($p = 0.001$) in ulnar loops in those whose mothers had received gamma globulin.

KEY WORDS Dermatoglyphics, congenital rubella, gamma globulin.

Patterns of palmar skin ridging are believed to attain their definitive configuration by the 19th week of fetal life (4). A fingerprint pattern which can be considered abnormal is very rarely seen in an individual patient but the altered relative distribution of the four main pattern types (whorls, ulnar loops, radial loops and arches) observed in certain groups of patients (6) presumably is a result of deviant embryologic development.

An increased percent of fingertips with whorl patterns has been observed in patients with the congenital rubella syndrome (1, 7, 8, 9) as has an increased frequency of individuals with whorls on eight, nine or ten fingertips (3, 7, 9). Our finding of a paucity of whorls in a small group of patients whose mothers had gestational rubella after having been given prophylactic gamma globulin prompted the present report.

CLINICAL MATERIAL AND METHODS

The fingertip skin ridge patterns of 39 children who had anomalies compatible with the congenital rubella syn-

drome were studied. All patients were born in the New York City area and the majority attended the Rubella Birth Defect Evaluation Project of New York University Medical Center. Rubella virus was isolated and/or there was serologic confirmation of the diagnosis in 7 instances. There was a physician's prospective clinical diagnosis of gestational rubella for eight of the twelve mothers of the remaining children.

The mothers of ten patients received gamma globulin (preparations unknown) because of exposure to rubella during pregnancy; all ten mothers nevertheless subsequently developed a rash. Their children will be referred to as having congenital rubella after gamma globulin in the other 29 patients as having congenital rubella (without prior gamma globulin). Cardiovascular anomalies, hearing impairment, mental retardation and neonatal purpura were present in both groups of patients but cataracts, birth weights below 2770 g, glaucoma and bone lesions were noted only in the children with congenital rubella (without prior gamma globulin).

The first four children in Table 1 were born in October 1955–January 1956, patient 19 in December 1963, patient 25 in January 1964 and the rest from July 1964–March 1965. Patients 1–16 and 30–33 were female. Patients 11, 12, 73 and 24 were Negro, patients 13–16 and 25–79 were Puerto Rican and the rest (including all in Table 2) were white.

A group of control children free of congenital anomalies was drawn from well baby and general pediatric clinics and a nursery school in New York City. There were 70 boys and 91 girls. Forty-two children were white, 37 Negro and 83 Puerto Rican. The birth weight of 35 children was under 27 kg.

Table 1 *Fingertip skin ridge patterns of those children with congenital rubella whose mothers did not receive gamma globulin before developing gestational rubella*

W=whorl U=ulnar loop R=radial loop A=arch * = no recognizable pattern X=poor print prevented analysis of pattern

Pt	Fingertip patterns	
	Right hand fingers 5 4 3 2 1	Left hand fingers 5 4 3 2 1
1	WWUUU	WWUUU
2	UUUUW	UUUUU
3	WUUUU	WWUUU
4	UUUUU	UUUUU
5	WWUUU	WWUUU
6	WWUUU	WWUUU
7	UUUUU	UUUUU
8	UUUUU	UUUUU
9	UUUUU	UUUUU
10	UUUUU	UUUUU
11	WWUUU	WWUUU
12	UUUUU	UUUUU
13	WWUUU	WWUUU
14	UUUUU	UUUUU
15	WWUUU	WWUUU
16	UUUUU	UUUUU
17	WWUUU	WWUUU
18	WWUUU	WWUUU
19	WWUUU	WWUUU
20	WWUUU	WWUUU
21	WWUUU	UUUUU
22	UUUUU	UUUUU
23	WWUUU	WWUUU
24	WWUUU	WWUUU
25	UUUUU	UUUUU
26	WWUUU	UUUUU
27	UUUUU	UUUUU
28	UUUUU	WWUUU
29	UUUUU	UUUUU

Fingerprints of the four children damaged in the 1955 epidemic were recorded. The skin of the fingertips of the remaining patients and of the control children was inspected through a 7× magnifying glass. All dermatoglyphic observations were made by one person.

The numbers of children with 0 1 2 10 whorls (hereafter referred to as the whorl profile) were determined as were the numbers of children with 0 1 2 10 ulnar loops (ulnar loop profile) the numbers with 0 1 2 10 arches (arch profile) and the numbers with 0 1 2 and 3 radial loops (radial loop profile). Each of the four pattern profiles of the children with congenital rubella after gamma globulin was compared with the corresponding profile of the children with congenital rubella (without prior gamma globulin) and the pattern profiles of each group of patients were compared with those of the control children. Inter group differences were evaluated statistically by the Mann-Whitney U test.

RESULTS

The ridge patterns of the patients are recorded in detail in Tables 1 and 2. The percentage distribution of pattern types on the fingertips of control children and patients is presented in Table 3. The frequencies of whorls and ulnar loops in both groups of patients with congenital rubella are outside the wide ranges of frequencies of these patterns observed in various Caucasian and Negro population samples (5).

Whorl and ulnar loop histogram profiles (7) of patients and control children are shown in Fig. 1. The statistically significant inter group differences in pattern profiles were:

a) Congenital rubella (without prior gamma globulin) compared with control children: whorl profile ($p < 0.001$), arch profile ($p < 0.01$) and ulnar loop profile ($p < 0.02$).

b) Congenital rubella after gamma globulin compared with control children: ulnar loop profile ($p = 0.001$) and whorl profile ($p = 0.01$). (Data limited to the white children showed a significant ($p < 0.01$) difference in ulnar loop and whorl profiles.)

c) Congenital rubella after gamma globulin compared with congenital rubella (without prior gamma globulin): whorl profile ($p < 0.001$), ulnar loop profile ($p < 0.001$) and arch profile ($p = 0.02$). (Data limited to the white children showed a significant ($p < 0.001$) difference in whorl and ulnar loop profiles.)

DISCUSSION

In the past, statistical evaluation of differences in the fingertip dermatoglyphics of two groups of individuals has usually been based on their pattern frequencies which in turn depend on the total numbers of fingertips with whorls with ulnar loops with radial loops and with arches. This method has been challenged by Berka, McClure, Sonley & Thompson (2) and by Preus & Fraser (6) because it assumes that the pattern on each fingertip of an individual is independently determined. Thompson &

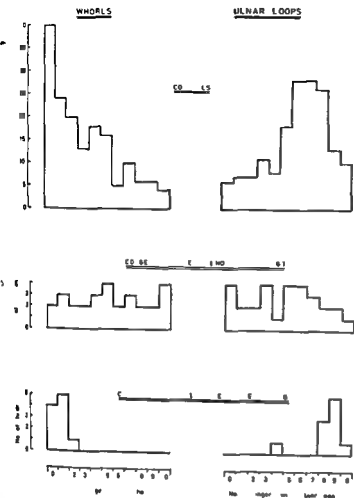


Fig 1 Whorl and ulnar loop histogram profiles of patients with congenital rubella and control children

Table 2 Fingertip patterns of those children with congenital rubella whose mothers received gamma globulin before developing gestational rubella

W=whorl U=ulnar loop R=radial loop A=arch

Pt	Gamma globulin			Fingertip patterns	
	Days after LMP	Amount (cc)	Days before rash	Right hand fingers 5 4 3 2 1	Left hand fingers 5 4 3 2 1
30	?	?	14	UUUUW	UUUUU
31	37	?	17	UUUUU	UUARU
32	40	11	7	UUUAU	UUUAU
33	57	70	7	UUARW	UUAAW
34	?	?	16	UUUUW	UUUUU
35	31	?	14	UUUUU	UUURU
36	37	20	6	UUUUU	UUUUW
37	68 and 86	10 and 10	24 and 24	UUUUU	UUUUU
38	75	16	15	UUUUU	UUUUU
39	77	70	2	UUUUU	UUUUU

Table 3 Frequencies of pattern types on the fingertips of control children and those with congenital rubella

	Control children	Children with congenital rubella	
		Mothers received gamma globulin before developing rubella	
		No	Yes
Number of children	162	29	10
Percent of fingertips with			
Whorls	31	52	7
Ulnar loops	60	46	83
Arches	7	0.3	6
Radial loops	2	1	4
Unknown		1	

Bandler (10) have demonstrated mathematically that this can not be so and that the patterns in any one individual tend to be alike. We suggest that it is more valid to compare the pattern profiles than the pattern frequencies of two groups since an individual is represented only once in a given pattern profile.

Comparison of the whorl profile of our patients (without prior gamma globulin) with that of the controls showed that the increase in whorls in these patients was indeed highly significant ($p < 0.001$) corroborating prior observations.

However the patients whose mothers had received gamma globulin before developing gestational rubella had significantly fewer whorls and more ulnar loops than the children

with congenital rubella (without prior gamma globulin) and unexpectedly more than the control children as well. The findings suggest that gamma globulin either alone or in interaction with rubella virus influenced fetal fingertip skin development.

REFERENCES

- 1 Alter M & Schulenberg R. Dermatoglyphics in the rubella syndrome. *JAMA* 197; 685 1966
- 2 Berka L, McClure P D, Sonley M J & Thompson M W. Dermatoglyphics in childhood leukemia. *Can Med Assoc J* 105: 476 1971
- 3 Gumpel S M. Clinical and social status of patients with congenital rubella. *Arch Dis Child* 47: 330 1972
- 4 Hale A R. Breadth of epidermal ridges in the human fetus and its relation to the growth of the hand and foot. *Anat Rec* 105: 763 1949
- 5 Plato C C, Cereghino J J & Steinberg F S. The dermatoglyphics of American Caucasians. *Am J Phys Anthropol* 42: 195 1975
- 6 Preus M & Fraser F C. Dermatoglyphics and syndromes. *Am J Dis Child* 174: 933 1977
- 7 Purvis Smith S G & Menser M A. Dermatoglyphics in adults with congenital rubella. *Lancet* II 141 1968
- 8 Purvis Smith S G & Menser M A. Genetic and environmental influences on digital dermatoglyphics in congenital rubella. *Pediatr Res* 7: 215 1973
- 9 Purvis Smith S G, Howard P R & Menser M A. Dermatoglyphic defects and rubella teratogenesis. *JAMA* 209: 1865 1969
- 10 Thompson M S & Bandler E. Finger pattern combinations in normal individuals and in Down's syndrome. *Hum Biol* 45: 563 1973

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RENAL FUNCTION IN INFANTS WITH HYPERBILIRUBINEMIA

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ABSTRACT Broberger U & Aperia A (Departments of Paediatrics at Karolinska Sjukhuset and St Goran's Children's Hospital Stockholm Sweden) Renal function in infants with hyperbilirubinemia. *Acta Paediatr Scand* 68 1979. A total of 45 infants were studied on the fourth or fifth day of life. 13 term and 10 pre-term infants with serum bilirubin levels ranging between 257 and 390 $\mu\text{mol/l}$ were compared with 12 term and 10 pre-term infants with serum bilirubin levels below 195 $\mu\text{mol/l}$. The groups did not differ with regard to mean gestational age or mean post-natal age. GFR and C_{PAH} were determined with the single injection clearance method and ability to excrete Na⁺ was determined following an oral loading of sodium chloride. GFR was lower in infants with hyperbilirubinemia and correlated negatively to the highest recorded serum bilirubin value. C_{PAH} was similar in hyperbilirubinemic infants and controls. The urinary sodium excretion was significantly higher in infants with hyperbilirubinemia.

KEY WORDS GFR, C_{PAH} , urinary sodium excretion, pre-term, full-term, hyperbilirubinemia.

Depressed renal function in the newborn infant is a normal state. The glomerular filtration rate is 25-30% of that in children and adults. The tubular capacity is also low. Hence diseases that do not primarily affect the kidney may still interfere with kidney function. This has been reported in infants with the idiopathic respiratory distress syndrome (3-7). In adults and animals with hyperbilirubinemia depressed renal function has been reported (1, 10, 12, 14, 16). The present study was undertaken to investigate the effect of unconjugated hyperbilirubinemia on glomerular filtration and sodium homeostasis in term and pre-term newborn infants.

MATERIALS

A total of 45 infants were studied on the fourth or fifth day of life. 13 term and 10 pre-term infants with hyperbilirubinemia were compared with 12 term and 10 pre-term infants without hyperbilirubinemia. The gestational age of the pre-term infants ranged between 33 and 36 weeks. The weights and lengths of the infants corresponded to their gestational ages. All pregnancies were uneventful. Five infants were delivered by caesarean section. 3 because of

breech presentation (one pre-term with and 2 without hyperbilirubinemia) and 2 because of narrow pelvis (one term with and one without hyperbilirubinemia). Apgar scores were 7 or more at 1 min and 8 or more at 5 min of age in all infants studied. The highest recorded serum bilirubin levels ranged between 260 and 390 $\mu\text{mol/l}$ in term infants and between 257 and 376 $\mu\text{mol/l}$ in pre-term infants. Conjugated bilirubin levels did not exceed 20 $\mu\text{mol/l}$. In term and pre-term controls the serum bilirubin was below 195 $\mu\text{mol/l}$. The clinical findings in term and pre-term infants with hyperbilirubinemia are shown in Table 1.

All infants received breast milk during the studies in an amount of 100-120 ml/kg BW/24 hrs. The pre-term infants were kept in Isolette incubators. The infants were disturbed as little as possible during the studies and showed no signs of discomfort. Informed parental consent was obtained in all cases. The protocol was approved by the Committee of Ethics at Karolinska Institute.

METHODS

Gestational age was determined with the method described by Dubowitz et al (5). Total serum bilirubin was determined in duplicate with a Bilirubinometer (American Optical Corp.). Conjugated bilirubin was determined with Nossing's method (11). Sodium in urine was analysed by a flame photometer (Eppendorf). Hematocrit was estimated in glass capillaries rinsed with heparin and centrifuged for 5 min at 12 000 g.

Table 3 Frequencies of pattern types on the fingertips of control children and those with congenital rubella

	Control children	Children with congenital rubella	
		Mothers received gamma globulin before developing rubella	
		No	Yes
Number of children	162	29	10
Percent of fingertips with			
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Comparison of the whorl profile of our patients (without prior gamma globulin) with that of the controls showed that the increase in whorls in these patients was indeed highly significant ($p < 0.001$) corroborating prior observations.

However the patients whose mothers had received gamma globulin before developing gestational rubella had significantly fewer whorls and more ulnar loops than the children

with congenital rubella (without prior gamma globulin) and, unexpectedly, than the control children as well. The findings suggest that gamma globulin either alone or in interaction with rubella virus influenced fetal finger skin development.

REFERENCES

- 1 Alter M & Schulenberg R. Dermatoglyphics in the rubella syndrome. *JAMA* 197; 685 1966
- 2 Berka L, McClure P D, Sonley M J & Thomson M W. Dermatoglyphics in childhood leukemia. *Can Med Assoc J* 105: 476 1971
- 3 Gumpel S M. Clinical and social status of infants with congenital rubella. *Arch Dis Child* 330 1972
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- 5 Plato C C, Cereghino J J & Steinberg F. The dermatoglyphics of American Caucasians. *J Phys Anthropol* 42: 195 1975
- 6 Preus M & Fraser F C. Dermatoglyphic syndromes. *Am J Dis Child* 124: 933 1971
- 7 Purvis Smith S G & Menser M A. Dermatoglyphics in adults with congenital rubella. *Lancet* II: 141 1968
- 8 Purvis Smith S G & Menser M A. Genetic and environmental influences on digital dermatoglyphics in congenital rubella. *Pediatr Res* 7: 215 1973
- 9 Purvis Smith S G, Howard P R & Menser M A. Dermatoglyphic defects and rubella teratogenesis. *JAMA* 209: 1865 1969
- 10 Thompson M S & Bandler E. Finger pattern combinations in normal individuals and in Down syndrome. *Hum Biol* 45: 563 1973

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Table 3 C_{PAH} gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
C_{PAH} (ml/1.73 m ² B S/min)	190 \pm 18.7	192.0 \pm 46.0	117.0 \pm 36.0	129.3 \pm 25.4
Gestational age (weeks)	39.0 \pm 0.0	40.8 \pm 0.8	35.0 \pm 0.0	34.4 \pm 1.8
Post natal age (hrs)	137.7 \pm 38.7	103.8 \pm 11.7	10.7 \pm 23.9	132.4 \pm 24.7
Hematocrit (%)	49.7 \pm 2.1	48.0 \pm 3.8	67.3 \pm 2.1	41.7 \pm 6.7
n	3	5	3	5

Values are means \pm S.D.

after administration of sodium chloride. The urinary sodium excretion was calculated as the average hourly excretion per 1.73 m² B S. Inulin in blood was determined using the Anthron method (8). PAH in blood was determined using the method described by Smith et al. (17).

RESULTS

The GFR C_{PAH} and sodium excretion in infants with hyperbilirubinemia and in controls are summarized in Tables 2, 3 and 4 respectively. The gestational age post natal age and hematocrit in the different groups are also shown.

Glomerular filtration rate

GFR was determined in 9 term infants with hyperbilirubinemia and 5 controls of the same post natal age and hematocrit. GFR was significantly lower in infants with hyperbilirubinemia i.e. 36.0 vs a vs 53.2 ml/1.73 m² B S/min in the controls (0.0025 > p > 0.0005).

GFR was also determined in 6 pre term infants with hyperbilirubinemia and in 5 con-

trols. Here the hyperbilirubinemic infants also had lower figures for GFR 31.1 vs a vs 38.3 ml/1.73 m² B S/min although the difference was not significant (0.10 > p > 0.05). There was a significant negative correlation between GFR and the highest serum bilirubin value recorded before the clearance study $r = -0.48$ (0.05 > p > 0.025). See Fig. 1.

Clearance of PAH

C_{PAH} was determined in 3 term and 3 pre term infants with hyperbilirubinemia and compared with 5 controls of similar gestational age and post natal age. No difference was found between hyperbilirubinemic infants and controls. The figures for term infants were 190.1 and 192.0 ml/1.73 m² B S/min respectively and for pre term infants 117.0 and 129.0 ml/1.73 m² B S/min.

Average hourly sodium excretion after an oral sodium load (UNaV)

UNaV was studied in 5 term infants with hyperbilirubinemia and 5 controls. UNaV was

Table 4 Average hourly sodium excretion UNaV gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
UNaV (mmol/1.73 m ² B S/h)	4.4 \pm 7.1	2.1 \pm 1.9	3.2 \pm 1.0	2.0 \pm 0.5
Gestational age (weeks)	39.8 \pm 0.5	40.0 \pm 1.0	35.7 \pm 0.8	34.4 \pm 1.1
Post natal age (hrs)	136.2 \pm 73.4	116.6 \pm 29.2	109.7 \pm 19.9	126.6 \pm 26.0
Hematocrit (%)	53.7 \pm 5.8	58.0 \pm 7.3	53.7 \pm 8.0	53.2 \pm 4.4
n	5	7	6	5

Values are means \pm S.D.

Table 1 Clinical data and post natal age at investigation in full term and pre term infants with hyperbilirubinemia

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Diagnosis	Serum bil $\mu\text{mol/l}$ /age hrs	Photo-therapy age hrs	Age at invest (hrs)
<i>Full terms</i>							
1	F	39	3 190	Phys jaund	308/ 88	72-120	155
2	M	39	2 940	ABO incomp	316/155	120-168	155
3	F	40	4 000	Phys jaund	387/ 86	73- 81	86
4	F	38	3 190	Phys jaund	351/100	84- 90	109
5	F	39	3 680	Phys jaund	356/100	40-134	110
6	F	39	2 700	ABO incomp	284/ 87	60-130	111
7	M	40	3 910	ABO incomp	308/ 71	77-100	107
8	M	40	3 400	ABO incomp	282/111	42- 80	111
9	F	42	3 250	Phys jaund	260/ 81	85- 99	109
10	F	40	2 940	Phys jaund	380/ 72	72-163	144
11	M	40	3 600	Phys jaund	375/ 75	78- 93	100
12	M	40	3 100	ABO incomp	390/143	126-170	155
13	M	40	3 650	Phys jaund	340/102	60-120	176
<i>Pre terms</i>							
1	M	34	2 270	Phys jaund	257/ 81	84-102	113
2	F	36	2 760	ABO incomp	376/ 75	77-150	134
3	F	35	2 100	Phys jaund	316/ 95	48-169	101
4	M	36	2 850	ABO incomp	333/104	100-170	119
5	M	35	2 790	ABO incomp	328/122	127-139	129
6	F	35	2 390	ABO incomp	342/ 76	18-144	76
7	F	36	2 680	ABO incomp	311/110	89-132	110
8	M	34	2 000	Phys jaund	354/ 88	85- 99	112
9	F	36	2 550	Phys jaund	272/120	104-140	144
10	M	36	2 890	ABO incomp	345/157	96-192	181

Single injection inulin and PAH clearance Inulin and PAH were injected i.v. in a dose of 100 and 15 mg/kg BW respectively. Calibrated glass syringes were used. Samples of the test solutions were kept for determination of inulin and PAH concentrations. Blood (0.4 ml) was collected before the test solutions were given and then every third minute from 7 to 19 min and every fifth minute from 45 to 70 or 90 min. The samples were collected in small test tubes rinsed with heparin and were kept on ice until they were centrifuged. The plasma was frozen and stored until analysed. The graphic resolution of the experimental curve was performed using the method described by

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The renal excretion of an oral sodium load (UNaV) was studied during standardized fluid expansion. Breast milk diluted with 50% water was administered by gastric tube in an amount corresponding to 2% of body weight during the first hour and then in an amount of 0.5% every 30 min. When a stable diuresis was established (50-60 mmol NaCl/1.73 m² B.S. (2-3 mmol/kg B.W.) was given orally in diluted breast milk. Urine was collected after spontaneous voiding in urinary bags for a time period of 4-6 hrs.

Table 2 GFR gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
GFR (ml/1.73 m ² B.S./min)	36.0 \pm 7.3	53.2 \pm 11.1	31.1 \pm 6.3	38.3 \pm 8.1
Gestational age (weeks)	39.6 \pm 1.1	40.8 \pm 0.8	35.0 \pm 0.6	34.4 \pm 1.8
Post natal age (hrs)	116.2 \pm 23.3	103.8 \pm 11.7	124.2 \pm 35.5	132.4 \pm 24.7
Hematocrit (%)	49.1 \pm 4.9	48.0 \pm 3.8	57.7 \pm 5.5	41.2 \pm 6.7
n	11	5	11	5

Values are means \pm S.D.

Table 3 C_{PAH} gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
C_{PAH} (ml/l 73 m ² B S /min)	190 \pm 18.7	197 \pm 46.0	117 \pm 36.0	129 \pm 25.4
Gestational age (weeks)	39 \pm 0.0	40 \pm 0.8	35 \pm 0.0	34 \pm 1.8
Post natal age (hrs)	132 \pm 38.7	103 \pm 11.7	107 \pm 23.9	132 \pm 24.7
Hematocrit (%)	49.7 \pm 2.1	48.0 \pm 3.8	67.3 \pm 2.1	41.2 \pm 6.7
n	3	5	3	5

Values are means \pm S.D.

after administration of sodium chloride. The urinary sodium excretion was calculated as the average hourly excretion per 1.73 m² B S. Inulin in blood was determined using the Anthron method (8). PAH in blood was determined using the method described by Smith et al. (17).

RESULTS

The GFR C_{PAH} and sodium excretion in infants with hyperbilirubinemia and in controls are summarized in Tables 2, 3 and 4 respectively. The gestational age, post natal age and hematocrit in the different groups are also shown.

Glomerular filtration rate

GFR was determined in 9 term infants with hyperbilirubinemia and 5 controls of the same post natal age and hematocrit. GFR was significantly lower in infants with hyperbilirubinemia i.e. 36.0 vs 53.2 ml/l 73 m² B S /min in the controls (0.0025 $> p >$ 0.0005).

GFR was also determined in 6 pre term infants with hyperbilirubinemia and in 5 con-

trols. Here the hyperbilirubinemic infants also had lower figures for GFR 31.1 vs 38.3 ml/l 73 m² B S /min although the difference was not significant (0.10 $> p >$ 0.05). There was a significant negative correlation between GFR and the highest serum bilirubin value recorded before the clearance study $r = -0.48$ (0.05 $> p >$ 0.025). See Fig. 1.

Clearance of PAH

C_{PAH} was determined in 3 term and 3 pre term infants with hyperbilirubinemia and compared with 5 controls of similar gestational age and post natal age. No difference was found between hyperbilirubinemic infants and controls. The figures for term infants were 190.1 and 192.0 ml/l 73 m² B S /min respectively and for pre term infants 117.0 and 129.0 ml/l 73 m² B S /min.

Average hourly sodium excretion after an oral sodium load (UNaV)

UNaV was studied in 5 term infants with hyperbilirubinemia and 7 controls. UNaV was

Table 4 Average hourly sodium excretion UNaV gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
UNaV (mmol/l 73 m ² B S /h)	4.4 \pm 2.1	2.1 \pm 1.9	3.2 \pm 1.0	2.0 \pm 0.5
Gestational age (weeks)	39.8 \pm 0.5	40.0 \pm 1.0	35.4 \pm 0.8	34.4 \pm 1.1
Post natal age (hrs)	136 \pm 23.4	116 \pm 19.7	109 \pm 19.9	116 \pm 26.0
Hematocrit (%)	53.2 \pm 5.8	58.0 \pm 7.3	53.2 \pm 8.0	43.2 \pm 4.4
n	5	7	6	5

Values are means \pm S.D.

Table 1 Clinical data and post natal age at investigation in full term and pre term infants with hyperbilirubinemia

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Diagnosis	Serum bil $\mu\text{mol/l}$ age hrs	Photo-therapy age hrs	Age at invest (hrs)
<i>Full terms</i>							
1	F	39	3 190	Phys jaund	308/ 88	72-120	155
2	M	39	2 940	ABO incomp	316/155	120-168	155
3	F	40	4 000	Phys jaund	387/ 86	73- 81	116
4	F	38	3 190	Phys jaund	351/100	84- 90	107
5	F	39	3 680	Phys jaund	356/100	40-134	110
6	F	39	2 700	ABO incomp	284/ 87	60-130	111
7	M	40	3 910	ABO incomp	308/ 71	77-100	107
8	M	40	3 400	ABO incomp	282/111	42- 80	111
9	F	42	3 250	Phys jaund	260/ 85	85- 99	109
10	F	40	2 940	Phys jaund	380/ 72	72-163	144
11	M	40	3 600	Phys jaund	325/ 75	78- 93	100
12	M	40	3 100	ABO incomp	390/143	126-170	155
13	M	40	3 650	Phys jaund	340/102	60-170	176
<i>Pre terms</i>							
1	M	34	2 270	Phys jaund	257/ 81	84-107	113
2	F	36	2 760	ABO incomp	376/ 75	77-150	134
3	F	35	2 100	Phys jaund	316/ 95	48-169	101
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5	M	35	2 790	ABO incomp	328/122	127-139	177
6	F	35	2 390	ABO incomp	342/ 76	18-144	76
7	F	36	2 680	ABO incomp	311/110	89-132	110
8	M	34	2 000	Phys jaund	354/ 88	85- 99	112
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Single injection inulin and PAH clearance Inulin and PAH were injected i.v. in a dose of 100 and 15 mg/kg B.W. respectively. Calibrated glass syringes were used. Samples of the test solutions were kept for determination of inulin and PAH concentrations. Blood (0.4 ml) was collected before the test solutions were given and then every third minute from 7 to 19 min and every fifth minute from 45 to 70 or 90 min. The samples were collected in small test tubes rinsed with heparin and were kept on ice until they were centrifuged. The plasma was frozen and stored until analysed. The graphic resolution of the experimental curve was performed using the method described by

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Table 2 GFR gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
GFR (ml/1.73 m ² B.S./min)	36.0 \pm 7.3	53.2 \pm 11.1	31.1 \pm 6.3	38.3 \pm 8.1
Gestational age (weeks)	39.6 \pm 1.1	40.8 \pm 0.8	35.0 \pm 0.6	34.4 \pm 1.8
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Hematocrit (%)	49.1 \pm 4.9	48.0 \pm 3.8	57.7 \pm 5.5	41.2 \pm 6.7
n	9	5	6	5

Values are means \pm S.D.

Table 3 C_{PAH} gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
C_{PAH} (ml/1.73 m ² B S/min)	190 ± 18.7	192.0 ± 46.0	117.0 ± 36.0	179.3 ± 25.4
Gestational age (weeks)	39.0 ± 0.0	40.8 ± 0.8	35.0 ± 0.0	34.4 ± 1.8
Post natal age (hrs)	132.7 ± 38.7	103.8 ± 11.7	102.7 ± 23.9	132.4 ± 74.7
Hematocrit (%)	49.7 ± 2.1	48.0 ± 3.8	67.3 ± 2.1	41.2 ± 6.7
n	3	5	3	5

Values are means ± S D

after administration of sodium chloride. The urinary sodium excretion was calculated as the average hourly excretion per 1.73 m² B S. Inulin in blood was determined using the Anthron method (8). PAH in blood was determined using the method described by Smith et al (17).

RESULTS

The GFR, C_{PAH} and sodium excretion in infants with hyperbilirubinemia and in controls are summarized in Tables 2, 3 and 4 respectively. The gestational age, post natal age and hematocrit in the different groups are also shown.

Glomerular filtration rate

GFR was determined in 9 term infants with hyperbilirubinemia and 5 controls of the same post natal age and hematocrit. GFR was significantly lower in infants with hyperbilirubinemia, i.e. 36.0 vs 53.2 ml/1.73 m² B S/min in the controls (0.0025 > p > 0.0005).

GFR was also determined in 6 pre term infants with hyperbilirubinemia and in 5 con-

trols. Here the hyperbilirubinemic infants also had lower figures for GFR, 31.1 vs 38.3 ml/1.73 m² B S/min, although the difference was not significant (0.10 > p > 0.05). There was a significant negative correlation between GFR and the highest serum bilirubin value recorded before the clearance study, $r = -0.48$ (0.05 > p > 0.025). See Fig. 1.

Clearance of PAH

C_{PAH} was determined in 3 term and 3 pre term infants with hyperbilirubinemia and compared with 5 controls of similar gestational age and post natal age. No difference was found between hyperbilirubinemic infants and controls. The figures for term infants were 190.1 and 192.0 ml/1.73 m² B S/min respectively and for pre term infants 117.0 and 129.0 ml/1.73 m² B S/min.

Average hourly sodium excretion after an oral sodium load (UNaV)

UNaV was studied in 5 term infants with hyperbilirubinemia and 7 controls. UNaV was

Table 4 Average hourly sodium excretion UNaV gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
UNaV (mmol/1.73 m ² B S/h)	4.4 ± 2.1	2.1 ± 1.9	3.7 ± 1.0	2.0 ± 0.5
Gestational age (weeks)	39.8 ± 0.5	40.0 ± 1.0	35.2 ± 0.8	34.4 ± 1.1
Post natal age (hrs)	136.2 ± 73.4	116.6 ± 29.7	109.7 ± 19.9	126.6 ± 26.0
Hematocrit (%)	53.2 ± 5.8	58.0 ± 7.3	53.2 ± 8.0	53.2 ± 4.4
n	5	7	6	5

Values are means ± S D

Table 1 Clinical data and post natal age at investigation in full term and pre term infants with hyperbilirubinemia

Pat no	Sex	Gest age (weeks)	Birth weight (g)	Diagnosis	Scrum bil $\mu\text{mol/l}$ age hrs	Photo-therapy age hrs	Age at invest (hrs)
<i>Full terms</i>							
1	F	39	3 190	Phys jaund	308/ 88	72-120	155
2	M	39	2 950	ABO incomp	316/155	120-168	155
3	F	40	4 000	Phys jaund	387/ 86	73- 81	86
4	F	38	3 190	Phys jaund	351/100	84- 90	109
5	F	39	3 680	Phys jaund	356/100	40-134	110
6	F	39	2 700	ABO incomp	284/ 87	60-130	111
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11	M	40	3 600	Phys jaund	325/ 75	78- 93	100
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13	M	40	3 650	Phys jaund	340/102	60-120	176
<i>Pre terms</i>							
1	M	34	2 270	Phys jaund	257/ 81	84-102	113
2	F	36	2 760	ABO incomp	376/ 75	77-150	134
3	F	35	2 100	Phys jaund	316/ 95	48-169	101
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6	F	35	2 390	ABO incomp	342/ 76	18-144	76
7	F	36	2 680	ABO incomp	311/110	89-132	110
8	M	34	2 000	Phys jaund	354/ 88	85- 99	117
9	F	36	2 550	Phys jaund	272/120	104-140	144
10	M	36	2 890	ABO incomp	345/157	96-192	181

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Table 2 GFR gestational age post natal age and hematocrit in infants with hyperbilirubinemia and controls

	Full terms		Pre terms	
	Hyperbil	Control	Hyperbil	Control
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Gestational age (weeks)	39.6 \pm 1.1	40.8 \pm 0.8	35.0 \pm 0.6	34.4 \pm 1.8
Post natal age (hrs)	116.2 \pm 23.3	103.8 \pm 11.7	124.2 \pm 35.5	132.4 \pm 24.7
Hematocrit (%)	49.1 \pm 4.9	48.0 \pm 3.8	57.7 \pm 5.5	41.2 \pm 6.7
n	9	5	6	5

Values are means \pm S.D.

ACKNOWLEDGEMENT

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REFERENCES

- 1 Aylward T H, Schowengerdt C E & Bove A. Experimental hyperbilirubinemia. Effect on glomerular filtration. *J Surg Res* 15: 1, 1973.
- 2 Broberger U. Determination of glomerular filtration rate in the newborn. *Acta Paediatr Scand* 62: 625, 1973.
- 3 Broberger U & Aperia A. Renal function in idiopathic respiratory distress syndrome. *Acta Paediatr Scand* 67: 313, 1978.
- 4 Calcagno P L & Rubin M I. Renal excretion of paraaminohippurate in infants and children. *J Clin Invest* 42: 1637, 1963.
- 5 Dubowitz L M S, Dubowitz V & Goldberg C. Clinical assessment of gestational age in the newborn infant. *J Pediatr* 77: 1, 1970.
- 6 Gekle D & Kuhner U. Untersuchungen über den nephrotoxischen Einfluss des Bilirubins auf die Nierenzellausscheidung Neugeborener. *Monatsschr Kinderheilk* 120: 304, 1977.
- 7 Guignard J P, Torrado A, Mazouni S M & Gautier E. Renal function in respiratory distress syndrome. *J Pediatr* 88: 845, 1976.
- 8 Hilger H H, Klumper J D & Ullrich K J. Wasserrückresorption und Ionentransport durch die Sammelrohrzellen der Säugetiermilch. *Pflügers Arch* 267: 218, 1958.
- 9 Klaus M H & Fanaroff A A. *Care of the High Risk Neonate*. W B Saunders, Philadelphia Pa, 1973, pp 332 & 337.
- 10 Mia A S, Gronwall H R, McGavin M D & Cornelius C E. Renal function defect in mutant South down sheep with congenital hyperbilirubinemia. *Proc Soc Exp Biol Med* 137: 1237, 1971.
- 11 Nossli H. The direct diazo-reaction of bile pigment in serum. *Scand J Clin Lab Invest* 12: suppl 49, p 1, 1960.
- 12 Odell G H, Natschka J C & Storey G N H. Bilirubin nephropathy in the Gunn strain of rat. *Am J Physiol* 212: 931, 1967.
- 13 Oh W & Karecki H. Phototherapy and insensible water loss in the newborn infants. *Am J Dis Child* 124: 730, 1977.
- 14 Powierza Kaczyńska C. Renal function in patients with viral hepatitis and obstructive jaundice. *Pol Med J* 11: 83, 1977.
- 15 Sapirstein L A, Vidich D G, Mandel M J & Hanusek G. Volumes of distribution and clearance of intravenously injected creatinine in the dog. *Am J Physiol* 181: 340, 1955.
- 16 Sitprija V. Renal function in jaundice. *J Med Assoc Thai* 53: 759, 1970.
- 17 Smith H W, Finkelstein N, Almenosa L, Crawford B & Graber M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin Invest* 24: 388, 1945.
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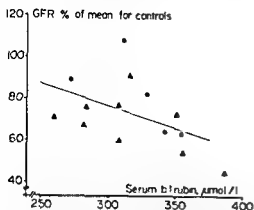


Fig 1 Relation between GFR and serum bilirubin level. GFR is expressed as % of the mean for three respective control groups: Preterm infants (○), Full term infants (●), and Controls (▲).

significantly higher in infants with jaundice 4.4, than in controls 2.1 mmol/l 73 m² B S/h (0.05 > *p* > 0.025). The same was found in 6 preterm infants with hyperbilirubinemia and 5 controls, i.e. 3.2 and 2.0 mmol/l 73 m² B S/h respectively (0.025 > *p* > 0.0125).

DISCUSSION

This study shows that the newborn infant with unconjugated hyperbilirubinemia has a reduced glomerular filtration rate. Phototherapy increases stool and insensible water losses (13) and may therefore influence the GFR since dehydration will result in a reduced GFR. In this study the hematocrit of the hyperbilirubinemic infants was within the normal range with regard to the postnatal age (9). The amount of fluid given should thus have compensated for the increased water loss. The reduction of GFR in the jaundiced infants could therefore be attributed to the hyperbilirubinemia as such. Thus, when the serum bilirubin exceeds 325 μmol/l the glomerular filtration rate is reduced to 65% of the expected normal value. In contrast to the GFR, the PAH clearance was not found to be reduced in jaundiced infants. It has been proposed that the development of the PAH transport is delayed due to a decrease either in tubular mass or tubular cell function

(4). The PAH clearance should in such a case underestimate the renal plasma flow. Provided that there is no difference in the PAH transport between infants with hyperbilirubinemia and control infants, hyperbilirubinemia should not affect the renal plasma flow. It is unlikely that infants with hyperbilirubinemia have an enhanced transport of PAH. If they have a decreased transport of PAH as compared with the control infants, the actual renal plasma flow would be higher than in the controls. In adults as well as in animals with hyperbilirubinemia of mixed conjugated and unconjugated type and in animals with unconjugated hyperbilirubinemia, a significant decrease in the glomerular filtration rate occurs (10, 14, 16). This has been attributed to a reduction in renal plasma flow as well as to a direct effect of bilirubin on glomerular function. The present results are more compatible with the latter explanation.

Signs of renal tubular impairment have previously been reported in unconjugated hyperbilirubinemia. These signs include enzymuria, decreased concentration capacity and sodium wasting (6, 12). In the present study both term and preterm infants with hyperbilirubinemia showed a higher sodium excretion after oral sodium loading than did their respective controls. Bilirubin is an uncoupler of oxidative phosphorylation in mitochondria (18). The enzymuria originating in the proximal tubular cells of newborn infants with unconjugated hyperbilirubinemia has been attributed to this effect (6). A similar mechanism may cause a decreased sodium transport and thus decrease the sodium reabsorption.

The reduced excretory function that has been shown in this study should have clinical implications for the pharmacological treatment of the hyperbilirubinemic newborn. This applies especially to the administration of aminoglycosides where a nephro- as well as a neurotoxic effect of the drug may be expected. The possibility of a negative sodium balance must also be considered during protracted hyperbilirubinemia in the newborn infant.

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REFERENCES

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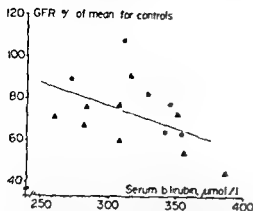


Fig 1 Relation between GFR and serum bilirubin level. GFR is expressed as % of the mean for three respective control groups: Preterm infants (●), Full term infants (▲), Controls (□).

significantly higher in infants with jaundice, 4.4 than in controls 2.1 mmol/l 73 m² B S/h (0.05 > p > 0.025). The same was found in 6 preterm infants with hyperbilirubinemia and 5 controls 1.3, 2.2 and 2.0 mmol/l 73 m² B S/h respectively (0.025 > p > 0.0125).

DISCUSSION

This study shows that the newborn infant with unconjugated hyperbilirubinemia has a reduced glomerular filtration rate. Phototherapy increases stool and insensible water losses (13) and may therefore influence the GFR since dehydration will result in a reduced GFR. In this study the hematocrit of the hyperbilirubinemic infants was within the normal range with regard to the postnatal age (9). The amount of fluid given should thus have compensated for the increased water loss. The reduction of GFR in the jaundiced infants could therefore be attributed to the hyperbilirubinemia as such. Thus when the serum bilirubin exceeds 325 μmol/l the glomerular filtration rate is reduced to 65% of the expected normal value. In contrast to the GFR the PAH clearance was not found to be reduced in jaundiced infants. It has been proposed that the development of the PAH transport is delayed due to a decrease either in tubular mass or tubular cell function

(4). The PAH clearance should in such a case underestimate the renal plasma flow. Provided that there is no difference in the PAH transport between infants with hyperbilirubinemia and control infants hyperbilirubinemia should not affect the renal plasma flow. It is unlikely that infants with hyperbilirubinemia have an enhanced transport of PAH. If they have a decreased transport of PAH as compared with the control infants the actual renal plasma flow would be higher than in the controls. In adults as well as in animals with hyperbilirubinemia of mixed conjugated and unconjugated type and in animals with unconjugated hyperbilirubinemia a significant decrease in the glomerular filtration rate occurs (10, 14, 16). This has been attributed to a reduction in renal plasma flow as well as to a direct effect of bilirubin on glomerular function. The present results are more compatible with the latter explanation.

Signs of renal tubular impairment have previously been reported in unconjugated hyperbilirubinemia. These signs include enzymatic decreased concentration capacity and sodium wasting (6, 12). In the present study both term and preterm infants with hyperbilirubinemia showed a higher sodium excretion after oral sodium loading than did their respective controls. Bilirubin is an uncoupler of oxidative phosphorylation in mitochondria (18). The enzymatic origin in the proximal tubular cells of newborn infants with unconjugated hyperbilirubinemia has been attributed to this effect (6). A similar mechanism may cause a decreased sodium transport and thus decrease the sodium reabsorption.

The reduced excretory function that has been shown in this study should have clinical implications for the pharmacological treatment of the hyperbilirubinemic newborn. This applies especially to the administration of aminoglycosides where a nephrotoxic as well as a neurotoxic effect of the drug may be expected. The possibility of a negative sodium balance must also be considered during protracted hyperbilirubinemia in the newborn infant.

MONONUCLEAR CELL MIGRATION INHIBITION IN CHILDREN WITH NEPHROTIC SYNDROME

K. KUCHARSKA, D. KOWALCZYK, K. SANCEWICZ-PACH and M. ZEMBALA

From the Institute of Paediatrics, Medical Academy, Kraków, Poland

ABSTRACT Kucharska K, Kowalczyk D, Sancewicz-Pach K and Zembala M (Institute of Paediatrics, Medical Academy, Kraków, Poland). Mononuclear cell migration inhibition in children with nephrotic syndrome. *Acta Paediatr Scand* 68: 81-84, 1979. — The relative number of T/B lymphocytes and the response of peripheral blood mononuclear cells to PHA in the migration inhibition test was studied in a group of patients with idiopathic nephrotic syndrome. It has been found that the level of T lymphocytes and the response to PHA was decreased in patients, in particular those with frequent relapses. The possible implications of these findings for pathogenesis of idiopathic nephrotic syndrome is briefly discussed.

KEY WORDS Nephrotic syndrome, T cell function, mononuclear cell migration inhibition.

The etiology of the idiopathic nephrotic syndrome with minimal changes in glomeruli is unknown. The response to steroid and cyclophosphamide therapy is considered evidence of an immunological background (3, 6) but against this are the normal blood levels of complement components including beta₂ globulin and the absence of glomerular deposits of immunoglobulins and C3. Also to be considered is the possibility of dysfunction of cell mediated immunity (7). It has been reported that children with minimal changes idiopathic nephrotic syndrome do not respond to sensitization with dinitrochlorobenzene (DNCB) (8). In addition, some of the affected children have a past history of allergic conditions (2).

Shalhoub (3) suggested that the T cells of nephrotic patients produce a lymphokine which is toxic for the glomerular basement membrane and increases its permeability to proteins leading to the development of nephrotic syndrome.

The present studies were designed to assess two aspects of cell mediated immunity in children with idiopathic nephrotic syndrome: the relative number of T lymphocytes and the response of peripheral blood mononuclear

(MN) cells to PHA in the migration inhibition test. As we have shown previously this test is a measure of T lymphocytes response to lymphokines produced by MN cells (10).

MATERIAL AND METHODS

Patients

15 children with idiopathic nephrotic syndrome aged from 19/12 to 11 years (mean 2.6/12) were studied. Six of these children were studied at the onset of the disease before prednisone therapy was started. The remaining patients were observed during second or subsequent relapses. Blood was taken at least 6 weeks after cessation of therapy. The standard therapy consisted of administration of prednisone 1–3 mg/kg/24h for a period of 3–8 weeks.

The clinical status of all the children was similar during the period of study. The patients were divided into 2 groups — 8 children with good response to prednisone therapy and 7 children with poor response to prednisone therapy relapsing over 4 times a year. 3 frequent relapsers. In three children from the second group renal biopsy was performed during the 6th or 8th relapses. Minimal changes in the glomeruli were present. Renal function tests were normal in all patients. Six healthy age matched children served as controls (Table 1).

Isolation of peripheral blood mononuclear cells and their surface markers

Mononuclear cells (MN) were isolated from the EDTA venous blood by standard Sodium Metrizoate Ficoll technique (5). T lymphocytes were scored by their ability to form rosettes with sheep erythrocytes (E-RFC) (9) with minor modifications (14).

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Patients

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The clinical status of all the children was similar during the period of study. The patients were divided into 2 groups: 8 children with good response to prednisone therapy and 6 children with poor response to prednisone therapy relapsing over 4 times a year. In frequent relapsers, in three children from the second group renal biopsy was performed during the 6th or 7th relapses. Minimal changes in the glomeruli were present. Renal function tests were normal in all patients. Six healthy age matched children served as controls (Table 1).

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Table 1 Clinical data of children with the nephrotic syndrome

No	Initials	Age	Sex	Age at the beginning of INS	Relapse	Laboratory findings			Renal biopsy
						Urinary protein (g/day)	Serum albumin (g/l)	Serum cholesterol (mmol/l)	
1	J I	2	F	2	I	4.5	12.3	10.0	
2	P M	19/12	F	18/12	I	33.7	13.2	16.1	
3	Cz M	2	F	2	I	33.0	13.6	8.1	
4	D K	3	M	3	I	4.2	13.3	10.0	
5	E A	2	M	2	I	16.3	14.1	9.5	
6	J G	6	M	6	I	3.0	16.2	10.7	
7	C B	6	F	3	II	3.2	16.3	9.3	
8	K R	3.5	M	14/12	III	3.8	10.3	17.2	
9	M R	5	F	2	IV	3.9	17.8	10.0	
10	N R	5	M	3	IV	12.4	14.2	9.7	
11	W J	8	M	6	IV	2.0	17.6	8.0	
12	T R	3.5	M	14/12	VI	2.5	18.0	8.0	
13	B K	3	F	17/12	VI	33.7	17.1	8.4	Minimal changes
14	K K	11.5	F	2	VII	28.0	24.0	6.0	Minimal changes
15	Z M	8	F	18/12	VII	2.5	10.4	8.0	Minimal changes

II lymphocytes bearing receptors for C3 were assessed by their ability to form rosettes with erythrocytes coated with IgM anti-sheep red cell antibody complement—EAC rosettes (4)

Statistical evaluation was done according to Student's *t* test

Migration inhibition test

Capillary tubes were filled with MN suspensions containing 3×10^7 cells/ml. After centrifugation the capillaries were cut at the fluid cell interface and placed into migration chambers plates (Sterilin). The tests were run in triplicates. Eagle's minimal essential medium supplemented with 10% foetal calf serum and antibiotics was used.

Purified phytohaemagglutinin (Wellcome Beckenham Kent, England) at the concentration 1 μ m/ml was used. In the control chambers PHA was omitted. For the details see (10). The migration index (MI) was calculated using the standard formula (2)

$$MI = \frac{\text{area of migration in the presence of PHA}}{\text{area of migration without PHA}}$$

RESULTS

Table 2 shows that the relative values of T lymphocytes were decreased in the patients as compared with the controls. This was more marked in the group with poor response to treatment. The level of B cells was increased in both groups of patients. These differences were however statistically non significant ($p > 0.05$).

Table 2 also shows that migration inhibition indices were higher i.e. the response to PHA was decreased in the patient group as compared to with the normal subjects. The re-

Table 2 The relative number of T and B lymphocytes and migration indices in children with INS

Group	No. of patients	Relative number of T lymphocyte ($\bar{x} \pm S.E.$)	Relative number of B lymphocyte ($\bar{x} \pm S.E.$)	Migration index ($\bar{x} \pm S.E.$)
Patients with nephrotic syndrome (total)	15	65.4 \pm 4.54	16.97 \pm 1.38	0.63 \pm 0.05
Group I	8	71.8 \pm 3.33	17.25 \pm 1.08	0.58 \pm 0.04
Group II	7	64 \pm 6.78	16.35 \pm 2.44	0.73 \pm 0.08
Control	6	75.66 \pm 1.47	13.25 \pm 2.34	0.47 \pm 0.04

$p < 0.05$

sponse of group II patients was decreased more than that of group I. The MI was remarkably high in 4 children 0.77, 0.96, 0.96 and 0.99 indicating no response to PHA. After prednisone therapy the values fell and reactivity to PHA was regained.

It was concluded that the response of patients' cells to PHA was depressed. This difference was statistically significant only for patients from group II.

DISCUSSION

Our results seem to indicate that cell mediated immunity is altered in the idiopathic nephrotic syndrome. Patients in the early stage of the disease did not show any significant changes while children experiencing many relapses showed a decreased number of T lymphocytes and poor production of lymphokines as judged from the results of migration inhibition. Decreased numbers of T lymphocytes in children with nephrotic syndrome were also found by Balish et al. (1).

The finding of a depressed response to PHA in the migration inhibition test is in keeping with observations that during the acute phase patients with idiopathic nephrotic syndrome have markedly decreased lymphocyte transformation to PHA (11). It is also interesting that delayed hypersensitivity skin reactions are depressed in nephrotic patients (8). Schulte-Wisserman et al. (12) postulated that in patients with idiopathic nephrotic syndrome circulating T suppressor cells are responsible for the depressed blast transformation to PHA. These cells may act via soluble factors. These suppressor cells may also be responsible for the depressed response in the migration inhibition test inhibiting a lymphokine production by patients' lymphocytes.

Our preliminary observations indicate that in patients whose cells did not respond to PHA in the migration inhibition test this ability was regained after therapy and clinical improvement. Similar results were obtained by Schulte-Wisserman et al. (12) in the lymphocyte

transformation test. They postulated that the effect of therapy is best explained by preferential action of steroids on T suppressor cells.

The role of depressed cell mediated immunity in the pathogenesis of INS is not clear. The disease may represent a T cell disorder. Our results support the notion that suppressor T cells and soluble factors produced by them may on one hand inhibit lymphocyte reactivity and on the other damage the glomerular basement membrane (12). This is in keeping with Shalhoub's (13) hypothesis.

At the practical level our results indicate that these simple tests of cellular reactivity may be clinically useful in monitoring the effect of therapy. Further and more detailed studies on a larger group of patients are needed to show whether the immunological mechanisms proposed are involved in the pathogenesis of the disease.

ACKNOWLEDGEMENTS

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REFERENCES

1. Bahah T, Neter E, Li P, K. Cohen E & Park B. H. Immunologic studies in minimal lesion nephrotic syndrome in children. Abstracts *Fourth International Symposium of Paediatric Nephrology* Helsinki, 1977: 81.
2. Balow J, Hurley D L & Fanci A. Immunosuppressive effects of glucocorticosteroids differential effects of acute vs chronic administration on cell mediated immunity. *J Immunol* 114: 1073 (1975).
3. Barrat T M & Soothill J F. Controlled trial of cyclophosphamide in steroid-sensitive relapsing nephrotic syndrome of childhood. *Lancet* II 479 (1970).
4. Bianco C, Patrick M & Nussenzweig V. A population of lymphocytes bearing a membrane receptor for antigen-antibody complement complex: Isolation and characterization. *J Exp Med* 137: 70 (1970).
5. Boyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 21 (Suppl 97): 51 (1968).
6. Cameron J S. Bright's disease today: The pathogenesis and treatment of glomerulonephritis. *Br Med J* IV 87 (1972).
7. Eyres K, Mallick N P & Taylor G. Leukocyte migration inhibition in minimal change nephropathy. *Lancet* II 198 (1976).

- 8 Halikowski B, Korczowski R & Zajczkowski J Odczyn skórny na dwinitrochlorobenzen (DNCB) u nosicieli antygenu Australia *Pediatr Pol* 47 1071 1972
- 9 Jondal M, Holm G & Wigzell H Surface markers on human T and B lymphocytes forming non immune rosettes with sheep red blood cells *J Exp Med* 136 207 1972
- 10 Kowalczyk D & Zembala M Migration inhibition of T lymphocytes from human peripheral blood by specific antigens and lymphokines *Clin Exp Immunol* 1978 (in press)
- 11 Moorthy A V, Zimmerman S W & Burkholder P M Inhibition of lymphocyte blastogenesis by plasma of patients with minimalchange nephrotic syndrome *Lancet* I 1160 1976
- 12 Schulte-Wissermann H, Lemmel E M, Rertz M, Beck J & Straub E Nephrotic syndrome of childhood and disorder of T cell function *Eur J Pediatr* 124 121 1977
- 13 Shalhoub R J Pathogenesis of lipoid nephrosis. A disorder of T cell function *Lancet* II 546 1974
- 14 Zembala M, Mytar B, Popiela T & Asherson G L Depressed in vitro peripheral blood lymphocyte response to mitogens in cancer patients: the role of suppressor cells *Int J Cancer* 19 605 1977

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PREDICTION OF ADULT HEIGHT WITH VARIOUS METHODS IN FINNISH CHILDREN

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From the Children's Hospital University of Helsinki Finland

ABSTRACT Lenko H L (Children's Hospital University of Helsinki Finland) Prediction of adult height with various methods in Finnish children *Acta Paediatr Scand* 68 1979 —Successive height predictions were made by several methods for a group of healthy Finnish children (30 boys and 30 girls) examined annually at ages of 7 to 17 years (1st series) and for 7 boys aged 11 to 19 years with familial delayed growth and puberty (2nd series). The methods used were those of Bayley & Pinneau (BP), Walker (W), Tanner et al. (T) and RWT and two simple principles: the relative height method (RH) which assumes constancy of height S D S throughout growth and the index of potential height (IPH) method which assumes constancy of height S D S for bone age (BA). The predictions with RH, W and IPH were inaccurate. BP, T and RWT were for the 1st series as accurate as for the basic series of the respective methods and none was superior to the others. The BA of average Finns was delayed as compared with the standards of Greulich Pyle Atlas. When corrections were made for this delay the IPH method gave predictions comparable in accuracy to BP, T or RWT. In the 2nd series prediction was more accurate with the corrected IPH, BP and RWT methods than with those using BA according to TW2RUS.

KEY WORDS Growth, height prediction, bone age

Several new methods have recently been developed for the prediction of adult height (9, 12, 14). Present height, weight, bone age, mid-parent height, growth velocity and pubertal stage are variably included in the prediction equations and so for the same child each method gives a somewhat different prediction. Applying these methods to series of children whose final heights are known should allow a comparative evaluation of the methods but no such study has previously been published. This is a report of such a comparison of the different methods in a series of healthy Finnish children.

MATERIALS AND METHODS

Subjects

Firstly predictions of adult height were made from data obtained yearly at ages 8-17 years for series of 30 boys and 30 girls, participants in a Finnish longitudinal growth study (5) whose heights at age 18 years were known. All were of Finnish ancestry, born in the Helsinki area and free from chronic disease. They had been seen yearly within one month of their birthdays. For calculations the

chronological age (CA) was rounded to whole years. Most of the subjects had missed one or more visits; the actual number of subjects of each sex seen at each age varied be-

List of abbreviations

CA	Chronological age
G BA	Greulich Pyle bone age
T BA	TW2RUS bone age
S D S	Standard deviation score

Methods for height prediction

RH	Relative height (assumes that the present and the final height S D S are equal)
IPH	Index of potential height (assumes that present and the final height S D S for bone age are equal)
IPH-G	IPH calculated at G BA
IPH-Gc	IPH calculated at corrected G BA
IPH T	IPH calculated at T BA
IPH Tc	IPH calculated at corrected T BA
W1	Walker method with height and CA
W2	Walker method with height, CA and growth rate
BP	Bayley Pinneau method
T1	Tanner et al. method with CA dependent coefficients
T2	Tanner et al. method with T BA dependent coefficients
RWT	Roche, Warner and Thussen method

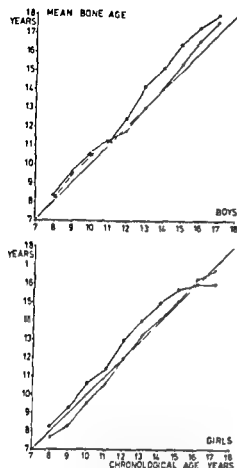


Fig 1 Mean Greulich Pyle (O) and TW 2 RUS (●) bone ages at different chronological ages in the first series of subjects. Identity is indicated by the diagonal

tween 16 and 78 (mean 23.5). Heights had been measured to the nearest 0.1 cm with the Martin anthropometer and weights to the nearest 0.1 kg. The measurements were always made by one of three observers. The heights at 18 years were taken as the adult heights, although some growth takes place after this age. Of the 18 boys and 20 girls whose later heights were known, 9 and 4 had grown >0.5 and 2 and 2 >1 cm, respectively. The mean adult heights were 177.5 cm \pm 5.9 cm (SD) and 164.1 cm \pm 5.1 cm, while the means reported for young Finnish males and females are 177.2 cm \pm 6.0 cm and 165.1 cm \pm 5.0 cm, respectively (2).

Secondly, similar predictions were made for a series of 7 healthy boys with familial delayed growth and puberty. They had been followed at our endocrine clinic and their adult heights were known. Full data for their total of 20 visits between the ages of 14 and 19 years were available.

Bone age

Bone age (BA) was determined from roentgenograms of hand and wrist taken at each visit. Both the Greulich Pyle Atlas (G BA) (4) and the TW 2 RUS method (T BA) (13) were used. For the G BA, the developmental stage of the epiphyses was compared with the standards, the carpal round bones being disregarded (1) and G BA was determined by interpolation to the nearest 0.25 years.

All BAs were determined independently by myself and another pediatrician, without knowledge of the identity or CA of the child. The mean of the two values was used in calculations. When they differed by >0.5 years (this occurred in 103 cases with G BA and 110 cases with T BA of the total of 469), we made repeat determinations without knowledge of the previous values. The mean of the second values was accepted even if the difference was again >0.5 years (24 and 31 cases, respectively). For T BA, no systematic difference was present between the two sets of readings. For G BA, one of us tended to obtain higher values than the other for girls; the mean difference was 0.1 years, which is statistically significant at the level $p < 0.001$.

Height prediction

Relative height method (RH) This assumes that the final height standard deviation score (SDS) (the deviation + or - in SD units from the mean for age and sex) will be the same as the present SDS. The SDSs were obtained from current standard growth curves for Finnish children based on a cross-sectional study (7).

Index of potential height methods (IPH) (7) These assume that the height SDS remains unchanged when calculated for BA (rather than for CA as in the RH method). IPH was calculated for both G BA (IPH G) and T BA (IPH T).

Walker methods (W) (14) W1 employs height and CA and W2, in addition, the growth rate over the preceding year. W3 was not used, because the necessary CA at peak height velocity was not always known.

Bayley and Pinneau method (BP) (3) This well-known method employs tables giving the percentages of final height that are acquired at each G BA for average, early and late maturers.

Tanner methods (T) (12) These equations contain coefficients for height, CA and T BA. The coefficients are specific for CA (method T1) or for T BA (T2). The equations that include midparent height were not used.

RWT method (9) This employs recumbent length, weight, midparent height and G BA. The recumbent length was obtained by adding 1.25 cm to the observed standing height (9). The tables are to be used only until half of the bones of the hand/wrist are mature (9). Our different usage of the Greulich Pyle standards did not allow such discrimination, and the tables were used up to CA 14 years for girls and 16 years for boys. For the second series, the coefficients for 16 years were used even at older age.

RESULTS

Bone age

The two methods gave different BAs. In girls and in pubertal boys, G BA was almost always lower than T BA. In prepubertal boys, individual differences were larger, but there was no systematic trend. In prepubertal girls, G BA tended to be lower than CA (Fig. 1), but the

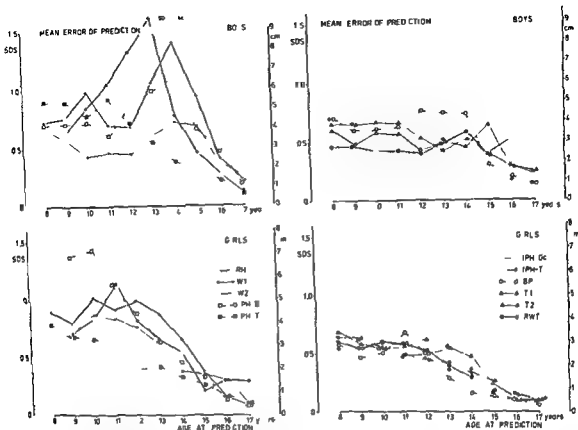


Fig 2 Mean absolute errors of yearly predictions by the different methods in the first series of subjects. For abbreviations see footnote on page 1

difference was not statistically significant. In boys the mean GBA was higher than CA except in 12–14 years and this difference was statistically significant at 10 and 17 years. The mean TBAs were almost consistently higher than CA and this difference was statistically significant at 10 and 12–15 years in girls and at 13–16 years in boys.

Accuracy of prediction

Fig 2 shows the mean of the absolute values of differences between the adult heights predicted and attained. Those methods of prediction that are based on CA and disregard earliness or lateness of maturation (i.e. methods RH and W) were inaccurate with the exception of W1 in boys. The use of BA instead of CA (IPH G and IPH T) improved the prediction only in the case of IPH T in girls. The more

elaborate methods BP T and RWT were significantly better. No clear differences were evident between the accuracies of these methods either individually or as based on GBA or TBA. The systematic errors were largest with RH, W and IPH. In general overprediction was more common for boys than for girls. Methods using TBA (IPH T, T1 and T2) gave overpredictions before puberty and underpredictions in puberty.¹

Consistency of predictions

For early evaluation of the effect of an intervention such as androgen treatment on final height consecutive predictions have to be

Detailed table of the statistics of systematic errors for each method, age and sex can be obtained at request from the author.

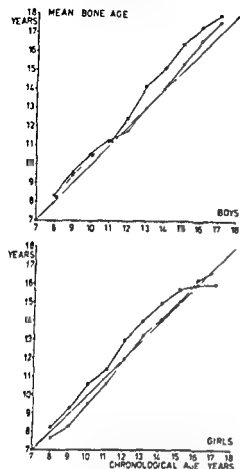


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RESULTS

Bone age

The two methods gave different BAs. In girls and in pubertal boys G BA was almost always lower than T BA. In prepubertal boys individual differences were larger, but there was no systematic trend. In prepubertal girls G BA tended to be lower than CA (Fig. 1), but the

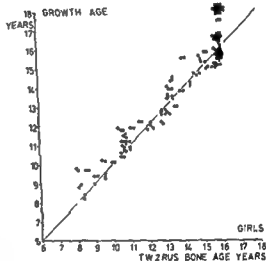
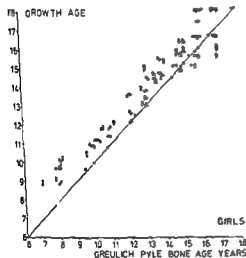
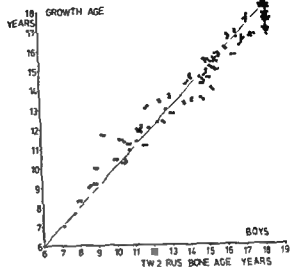
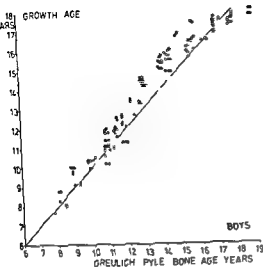


Fig 4 Bone age plotted against corresponding growth age (age at which the actual would equal the final height

S D S) in the first series of subjects. Identity is indicated by the diagonal

T In the T methods the BA is not given enough weight in situations where the difference between CA and BA is large

DISCUSSION

The present observations are fairly close to those made in the original series of the developers of the various methods. The W methods gave quite large errors. W1 gave reasonably good predictions for boys but not for girls and W2 was poor for both sexes. This may be due to differences between the ob-

served populations of New Haven and Finland in timing of growth and in final height. Different timing of pubertal growth and the imprecision of the estimates of growth rate (which cumulates the imprecisions of two height measurements) presumably explain the lesser accuracy of W2 as this includes growth rate. With BP the systematic errors were slightly larger in my series than in the basic and validating series of Bayley and Pinneau. BP tended to give overprediction especially for the boys. The dispersion of the errors was wider in my first series than in Bayley and Pin-

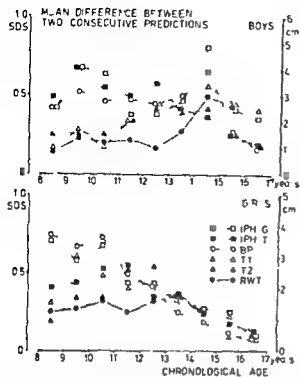


Fig. 3 Mean differences between each two consecutive yearly predictions by the different methods in the first series of subjects. For abbreviations see footnote on p. 1.

compared. The reliability of such evaluations has to be judged in terms of the consistency of consecutive predictions in the absence of any intervention. The mean differences between two consecutive predictions for the first series are shown in Fig. 3 for methods BP, T, RWT, IPH G and IPH T. The consistency is relatively poor, especially for the methods in which the influence of BA is greatest (BP, IPH).

Correction of bone age

RH gave overpredictions at all ages. The systematic error was largest at ages just before the steepest pubertal part of the Finnish standard growth curves. Clearly the pubertal growth spurt occurred earlier in the present series than in the average Finnish children on whom the standards were based. The G BAs are not so advanced over CA (Fig. 1). Growth age, the age at which the child's actual height SDS would have been identical with his or her final SDS, was calculated to the nearest 0.1 year for each child at each CA. These growth ages were plotted against the corre-

sponding BAs (Fig. 4). The G BAs were systematically higher than the corresponding growth ages, but the dispersion was smaller than with the T BAs, which agreed better with the growth ages. The BA growth age pairs were classed separately for boys and girls, firstly according to BA and secondly according to growth age. Full years were used as class limits. The mean growth age was calculated for each BA class and the mean BA for each growth age class. All these means were then plotted, growth age versus BA. A curve smoothed by eye was drawn through these plots (Fig. 5). These curves were used for correction of BAs, the growth age being taken for the corrected BA. These curves show that the average Finnish children on whom the standards were based were delayed in their skeletal maturation as compared with the Greulich-Pyle standards, especially at puberty. Compared with the TBA standards, no such difference was present for boys, but girls under 13 years old showed a slight advance.

Effect of correction of BA on the accuracy of prediction

The individual BAs were corrected for the systematic error according to Fig. 5. With the G BA so corrected, IPH had a markedly improved accuracy (see Fig. 2, IPH Gc). Correction of TBA did not similarly increase the accuracy of IPH T. The IPH methods with the corrected BAs were similar in consistency to BP.

Height prediction in boys with delayed puberty

The G BAs of these boys lagged behind CA by 2–5 years and the T BAs by 0.5–5 years. Fig. 6 shows the mean absolute errors of the first predictions made with different methods for each boy. Predictions based on later data did not differ significantly from the first predictions. In these boys, the G BA seemed to give a more correct measure of maturity than the TBA, as the methods IPH Gc, BP and RWT were all more accurate than IPH T and

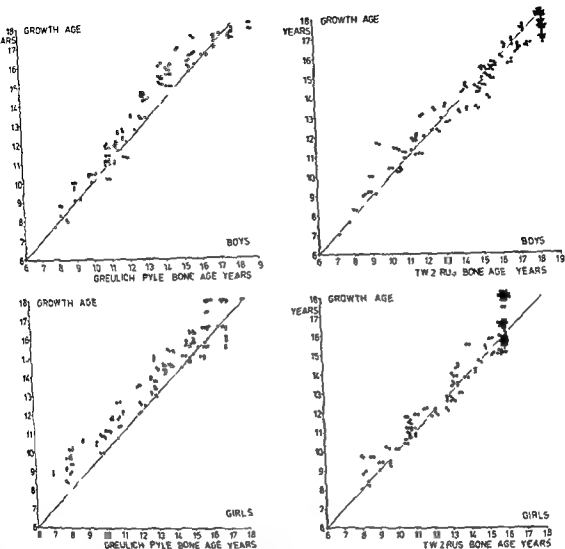


Fig 4 Bone age plotted against corresponding growth age (age at which the actual would equal the final height)

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DISCUSSION

The present observations are fairly close to those made in the original series of the developers of the various methods. The W methods gave quite large errors. W1 gave reasonably good predictions for boys but not for girls and W2 was poor for both sexes. This may be due to differences between the ob-

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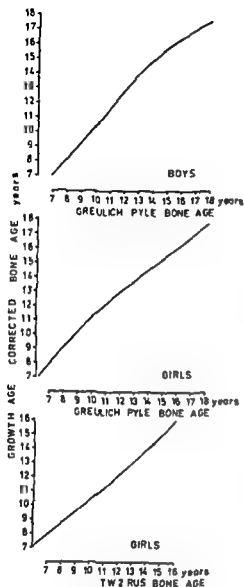


Fig 5 Correction curves for bone age according to the average growth of Finns (see text)

ner's basic sample is expected but it was usually smaller than in their validating sample. With the T methods the SDs of the errors were comparable to the residual SDs found by Tanner. The girls of 9–12 years in my series were less accurately predicted than the ballet dancers of Tanner but the difference is not statistically significant. With RWT the median absolute error and the 90th percentile of absolute error of prediction did not greatly differ in my series as compared with the children in the original Fels growth study. In the Finnish sample the predictions showed a larger error when based on data for both boys and girls under 10 years but at later ages were even

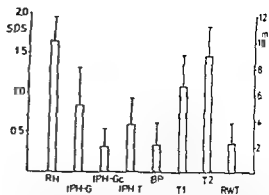


Fig 6 Absolute errors (mean + 2 SD) in height prediction by the different methods in boys with familial delayed growth and puberty (second series of subjects). For abbreviations see footnote on p 1

more accurate than those in the original series. Our modified method for determining GBA may explain the slight systematic overprediction for both sexes obtained with RWT. Yet with BP using the same BA, girls were systematically underpredicted. Thus it appears that although different populations differ in the timing of growth, the methods which use BA in addition to CA give as good an accuracy for Finnish children as for American or English children.

Children are said to have a tendency to follow their own genetic growth channels (6). But this rule applied in our RH method did not lead to good predictions of adult height. It was surprising how little these predictions were improved by using BA to allow for differences in timing of growth. One source of error is that the Finnish growth standards are cross sectional rather than longitudinal and thus do not present the actual shape of growth curves at puberty (11). Further, the average Finn differs in the timing of growth and maturation from the populations on which the BA standards were based and the BAs must be corrected to Finnish growth standards as I have now done.

One of the main sources of inaccuracy in prediction of height is the inaccuracy of the BA estimation. This estimation is always somewhat subjective. With experience the observer reduces the variance of his estimates but between observer differences cannot be

avoided (1). A small difference in BA determination can lead to a great difference in height prediction especially during the pubertal spurt. The G BA was somewhat more reproducible than the T BA according to comparison between my two observers' readings. The T BA places much weight on the maturation of some bones and a one stage difference can cause a year's difference in BA. No one knows which criteria of bone maturation are the most significant for prediction of height. Neither is it clear whether the hand/wrist BA corresponds best to growth as different parts of the skeleton differ in maturation. The knee has been suggested to be the most informative for assessing growth potential (10) but height prediction methods based on the knee BA have not been published.

The different methods of height prediction have a similar accuracy but require a very variable amount of calculation. G BA is easier to determine than T BA. The T BA is also subject to calculation errors. The IPH methods are rapid and adequate. The BA scales should be checked and if necessary corrected for every population. For Finnish children T BA needed less correction than G BA. Therefore IPH Tc might seem better for Finns than IPH Gc. However IPH Tc shows greater variance and a tendency to overprediction before puberty and underprediction during puberty and so may lead to serious misinterpretations of the effects of interventions. BP is very simple and gives reasonably accurate predictions. The T methods involve more cumbersome calculations especially if the coefficients are extrapolated to the exact age as suggested by the authors. In healthy children T1 and T2 agreed well. RWT seems to be the most accurate but involves the greatest amount of calculations; its inaccuracy increased with age and it should be used only up to the BA stated by its authors. Both with RWT and with T the predictions may be less than the actual height already achieved.

It is not known whether these methods are applicable to children with growth disorders

and at the extremes of normal variation. Yet it is in these situations that height predictions are most commonly and most urgently needed. BP and T1 have been reported to be accurate in tall girls (8) (15) and T1 in tall boys (16) but the number of observations was small. In our group of boys with delayed puberty appropriate predictions were obtained with IPH BP and RWT but not with T.

ACKNOWLEDGEMENT

I am indebted to Dr R. L. Kantero for allowing me to use for this study the longitudinal data on normal children collected by the Finnish Centre for Study in Child Growth and Development.

REFERENCES

1. Acheson R M, Fowler G, Fry E I, Janes M, Koski K, Urbano P & van der Werff Ten Bosch J J. Studies in the reliability of assessing skeletal maturity from X rays. Part I. Greulich Pyle Atlas. *Hum Biol* 37: 317, 1963.
2. Backstrom L & Kantero R L. Cross sectional studies of height and weight in Finnish children aged from birth to 20 years. *Acta Paediatr Scand* Suppl 240: 9, 1971.
3. Bayley N & Pinneau S R. Tables for predicting adult height from skeletal age: revised for use with the Greulich Pyle hand standards. *J Pediatr* 40: 423, 1957.
4. Greulich W W & Pyle I S. *Radiographic Atlas of Skeletal Development of the Hand and Wrist*. Stanford University Press, Stanford, 1959. 2nd ed.
5. Hallman N, Backstrom Jarvinen L, Kantero R L & Tussala H. Finnish centre for study in child growth and development. *Ann Paediatr Fenn* 12: 8, 1966.
6. Kantero R L, Tussala R & Backstrom L. Predictability of individual height and weight growth from cross sectional distance curves from birth to 10 years. *Acta Paediatr Scand* Suppl 240: 33, 1971.
7. Perheentupa J, Lenko H L, Nevalainen I, Nuttymaki M, Soderholm A & Taipale V. Hormonal therapy in Turner's syndrome: Growth and psychological aspects. In *Proceedings of the XIV International Congress of Pediatrics* 5. Buenos Aires, 1971. p. 121.
8. Roche A F & Wetenhall H N B. The prediction of adult stature in tall girls. *Aust Paediatr J* 5: 13, 1969.
9. Roche A F, Warner H & Thissen D. The RWT method for the prediction of adult stature. *Pediatrics* 56: 1076, 1975.
10. Roche A F, Warner H & Thissen D. *Skeletal maturity: The knee joint as a biological indicator*. Plenum Medical Book Company, New York, 1976.

- 11 Tanner J. M. *Growth at adolescence*. Blackwell Scientific Publications, Oxford 1962. 2nd ed.
- 12 Tanner J. M., Whitehouse R. H., Marshall W. A. & Carter B. S. Prediction of adult height from height bone age and occurrence of menarche at ages 4 to 16 with allowance for midparent height. *Arch Dis Child* 50: 14, 1975.
- 13 Tanner J. M., Whitehouse R. H., Marshall W. A., Healy M. J. R. & Goldstein H. *Assessment of skeletal maturity and prediction of adult height TW2 Method*. Academic Press, New York 1975.
- 14 Walker R. N. Standards for somatotyping children. I. The prediction of young adult height from children's growth data. *Ann Hum Biol* 1: 14, 1974.
- 15 Zachmann M., Ferrandez A., Murset G. & Prader A. Estrogen treatment of excessively tall girls. *Helv Paediatr Acta* 30: 11, 1975.
- 16 Zachmann M., Ferrandez A., Murset G., Gnehm H. E. & Prader A. Testosterone treatment of excessively tall boys. *J Pediatr* 88: 116, 1976.

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SERIAL DETERMINATIONS OF SERUM FERRITIN IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

Evaluation of its Usefulness as a Prognostic Index

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ABSTRACT Koller ME Romslo I Finne PH and Haneberg B (Laboratory of Clinical Biochemistry Department of Paediatrics University of Bergen Bergen Norway) Serial determinations of serum ferritin in children with acute lymphoblastic leukemia *Acta Paediatr Scand* 68 93 1979. —Thirty children with acute lymphoblastic leukemia were monitored with serial serum ferritin determinations for up to 17 months. In children with acute lymphoblastic leukemia before initiation of therapy or in relapse the mean serum ferritin concentration was 636 $\mu\text{g/l}$. In children who went into primary remission the mean serum ferritin concentration fell from 265 $\mu\text{g/l}$ prior to start of treatment to 161 $\mu\text{g/l}$ after 3 months of treatment. Five patients relapsed. Their serum ferritin levels prior to the relapses ranged from 7 to 135 $\mu\text{g/l}$. At the time of relapse a further increase in serum ferritin was found in only 2 of the children. Thus whereas high serum ferritin levels may signal disease activity in acute lymphoblastic leukemia a normal serum ferritin level does not exclude disease activity or impending relapse.

KEY WORDS Acute lymphoblastic leukemia serum ferritin

Determination of serum ferritin is a simple way of quantifying the body iron stores in normal subjects and in patients with iron deficiency or iron overload (1, 5, 7, 10). However in a number of conditions a marked increase in serum ferritin occurs that is unrelated to the iron stores. Thus high levels inappropriate for the amount of iron storage have been found in patients with liver diseases, tumour bearing patients and in patients with leukemia (2, 4, 6–8). It has been suggested that the high concentrations of ferritin in these patients are related to an abnormal production and release of ferritin together with reduced plasma clearance (2).

This report deals with serial determinations of serum ferritin in infants and children with acute lymphoblastic leukemia during a 17 month period. Data on serial determinations of ceruloplasmin, orosomucoid and alanine aminotransferase activity are also included. The aim of the present study was to evaluate

the usefulness of the serum ferritin concentration as a prognostic index in childhood leukemia.

MATERIALS AND METHODS

Sera were obtained at monthly intervals for up to 17 months from 30 infants and children with acute lymphoblastic leukemia (ALL). The following patients were studied:

8 girls and 5 boys aged 5–15 years with ALL in hematological remission after cessation of therapy. On entering the study they had been off therapy for from 4 months to 4 years.

5 girls and 1 boy aged 8 months–13 years with ALL on therapy. Twelve of these patients were studied from the time of diagnosis, i.e. before treatment. Four patients entered the study during treatment while in hematological remission and 1 entered the study while in relapse.

Conventional chemotherapy was used. Induction of remission in ALL was usually achieved by vincristine and prednisone or dexamethasone followed by asparaginase and later by high doses of methotrexate intravenously. Maintenance therapy with 6-mercaptopurine plus methotrexate and reinduction courses of vincristine and steroids every 7–3 months were continued for 3 years. In cases of relapse cytosine arabinoside and cyclophosphamide were added. All patients received methotrexate intrathecally.

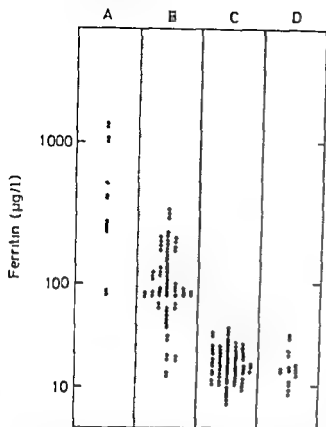


Fig. 1 Serum ferritin concentrations in children with acute lymphoblastic leukemia (A) before treatment and on treatment while in incomplete remission or relapse (B) on therapy while in remission (C) after cessation of therapy. The concentrations in healthy children (D) are also shown.

Radiation therapy was restricted to patients with leukemic involvement of the central nervous system or with testicular infiltrations.

The serum concentrations of ferritin were measured by a solid phase radioimmunoassay kit (from Ramco Lab Inc Houston Texas USA) using antihuman spleen ferritin. This kit has been shown to give values slightly lower than those obtained by other methods (M Worwood personal communication).

Ceruloplasmin and orosomucoid were determined by standard immunoplate techniques (Behringwerke AG Marburg Lahn W Germany).

Alanine aminotransferase (ALAT) was determined by the method recommended by The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (3). All determinations were run in duplicate.

Our reference values (mean \pm 2 S D) determined on 31 healthy children between 2–14 years of age were 3–31 μ g/l, 0.20–0.40 g/l, 0.40–0.80 g/l and 20–40 U/l for ferritin, ceruloplasmin, orosomucoid and ALAT respectively. The day to day coefficients of variation of the determinations were ferritin 6.9%, ceruloplasmin 7.0%, orosomucoid 7.7% and ALAT 6.0%.

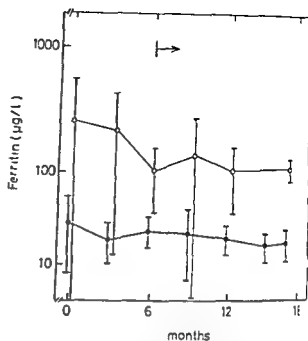


Fig. 2 Serial determinations of serum ferritin in 13 long-term survivors of childhood acute lymphoblastic leukemia off therapy (●) and in 10 children with acute lymphoblastic leukemia responding favourably to treatment at presentation and during treatment (○). The results are given mean \pm S D. At the point indicated (arrow) all the patients on treatment had normal bone marrow smear.

RESULTS

In patients with pathological marrow smear either on or off therapy the mean serum ferritin concentration was 636 μ g/l (range 49–4945 μ g/l from 38 observations in 16 patients) (Fig. 1). On the other hand, in patients in remission and with a normal bone marrow the mean serum ferritin concentration was 133 μ g/l (range 7–610 μ g/l from 97 observations in 13 patients). Children previously treated for ALL and still in remission had mean serum ferritin concentration 23 μ g/l (range 4–100 μ g/l from 96 observations in 11 patients).

Among children who responded favourably to therapy ($n=10$) the remission coincided with a fall in the serum ferritin concentration (Fig. 2). The mean pretreatment value was 265 μ g/l (range 49–1300) and after 3 months of successful therapy the mean value was 161 μ g/l (range 35–364 μ g/l). Corresponding

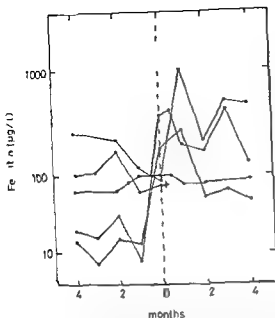


Fig 3 Serial determination of serum ferritin in children with acute lymphoblastic leukemia in remission and during relapse. At the point indicated (stippled line) the patients were in relapse with pathological bone marrow

figures for ALAT were 33 and 66 U/l respectively

The serum ferritin concentrations in 5 patients who relapsed did not increase prior to the appearance of pathological bone marrow (Fig 3)

The serum ferritin concentration showed small fluctuations during infections and did not correlate with the concentrations of orosomucoid and ceruloplasmin. Furthermore there was no correlation between serum ferritin and ALAT (data not shown)

DISCUSSION

The high serum ferritin concentrations found in children with ALL before treatment or during relapse are in agreement with previous reports (4-8). The values were 10-30 times higher than those found in healthy children (7). On the other hand, our results in children in remission on therapy differ from those re-

ported by Parry et al (6) who found a marked increase in serum ferritin concentrations during induction of primary remission. Our findings suggest that following chemotherapy an uneventful remission coincides with a fall in serum ferritin concentration. This is also supported by the findings of Sumes et al (8) who found that the serum ferritin values in children with ALL during remission were significantly lower than at initial diagnosis.

It should be noted that increasing serum ferritin levels did not precede relapse of ALL. In fact increasing serum ferritin levels were not found until after the relapse had been diagnosed by conventional marrow smears.

Such hyperferritinemia might be ascribed to an increased ferritin synthesis by the leukemic cells (11) together with an increased release from damaged leukemic cells (6). This assumption has been challenged by Sumes et al (8) who found no increase in the serum ferritin on rapid lysis of leukemic cells following chemotherapy. Our results favour those reported by Sumes et al (8).

In liver diseases hyperferritinemia has been ascribed to decreased removal of ferritin from the circulation by damaged hepatic parenchymal cells (2, 8). As shown by Parry et al (6) and confirmed in the present study in leukemia there was no correlation between serum ferritin concentration and transaminase activity, i.e. the high ferritin levels did not result from liver damage.

Serum ferritin is frequently elevated in infections and inflammations irrespective of the iron status of the patient (> 7). This may suggest that the hyperferritinemia in ALL could be an acute phase reaction (8, 9). However the fact that our results fail to show any correlation between serum ferritin concentration and ceruloplasmin or orosomucoid in children with ALL would suggest that the high ferritin levels are not the results of an acute phase reaction.

Our findings indicate that in patients with ALL determination of serum ferritin may be useful in assessing the state of remission but

it is of dubious value in the early detection of relapses

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REFERENCES

- 1 Jacobs A, Miller F, Worwood M, Bermish M R & Wardrop C A. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br Med J* 11: 206, 1972.
- 2 Jacobs A. Serum ferritin and iron stores. *Fed Proc* 36: 2024, 1977.
- 3 Weiding R, Horder M, Gerhardt W, Pitkanen E, Tenhunen K, Strømme J H, Theodorsen L, Waldenström J, Tryding N & Westlund L. Recommended methods for the determination of four enzymes in blood. *Scand J Clin Lab Invest* 33: 291, 1974.
- 4 Jones P A E, Miller F M, Worwood M & Jacobs A. Ferritinemia in leukemia and Hodgkin's disease. *Br J Cancer* 27: 212, 1973.
- 5 Lipschitz D A, Cook J D & Finch C A. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med* 290: 1213, 1974.
- 6 Parry D H, Worwood M & Jacobs A. Serum ferritin in acute leukemia at presentation and during remission. *Br Med J* 1: 245, 1974.
- 7 Sumes M A, Addiego J E & Dallman P R. Ferritin in serum: Diagnosis of iron deficiency and iron overload in infants and children. *Blood* 43: 481, 1974.
- 8 Sumes M A, Wang W C & Dallman P R. Elevated serum ferritin in children with malignancies. *Scand J Haematol* 19: 153, 1977.
- 9 Thomas W J, Koenig H M, Lightsey Jr A L & Green H. Free erythrocyte porphyrin, hemoglobin ratios, serum ferritin and transferrin saturation levels during treatment of infants with iron-deficiency anemia. *Blood* 49: 455, 1977.
- 10 Walters G O, Miller F M & Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 26: 770, 1973.
- 11 Worwood M, Summers M, Miller F, Jacobs A & Whittaker J A. Ferritin in blood cells from normal subjects and patients with leukemia. *Br J Haematol* 28: 27, 1974.

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INFLUENCE OF GENERAL ANAESTHESIA ON ANO-RECTAL MANOMETRY IN HEALTHY CHILDREN

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ABSTRACT Frickner B & Molander M-L (The Department of Paediatric Surgery, St Goran's Hospital, Stockholm, Sweden). Influence of general anaesthesia on ano-rectal manometry in healthy children. *Acta Paediatr Scand* 68 97 1979.—According to several investigations ano-rectal manometry is a valuable diagnostic test of Hirschsprung's disease. In order to yield accurate results it requires a quiet, calm child who cooperates. In the few instances when this is not possible, general anaesthesia may be desirable. Manometric recordings of the internal anal sphincter activity were therefore performed in 11 healthy children when awake and during general anaesthesia. The tonic activity at rest was significantly reduced during anaesthesia. Relaxations of the internal sphincter in response to rectal distension were recorded in all children both when awake and during anaesthesia. They were however significantly less pronounced during anaesthesia. These findings strongly suggest that ano-rectal manometry in the diagnosis of Hirschsprung's disease may be performed with advantage during general anaesthesia if the child does not cooperate when awake.

KEY WORDS Ano-rectal manometry, Hirschsprung's disease, general anaesthesia.

Like other smooth muscle, the internal anal sphincter is in a state of continuous tonic activity and normally keeps the anal canal closed. When the rectum is distended, however, there is a relaxation of this sphincter (2, 9, 13) which results in a fall in anal pressure. This recto-sphincteric reflex is mediated via local nerve pathways in the wall of the gut and is independent of spinal cord connections (2). When the ganglion cells of the rectum are absent, however, as in Hirschsprung's disease, the internal sphincter fails to relax upon rectal distension (11, 12). Several authors have found this to be a valuable diagnostic test of Hirschsprung's disease (1, 6, 10, 14, 15, 16). To obtain conclusive recordings it is essential that the patient is quiet and calm, which is mostly the case. If the child does not cooperate, however, general anaesthesia may be desirable, but it is not yet known how this can influence the normal recto-sphincteric reflexes.

The aim of the present investigation was to study the influence of general anaesthesia on anal pressure and recto-sphincteric reflexes in healthy children.

MATERIAL

The study was undertaken on 15 healthy children admitted to the hospital for a minor operation such as circumcision or herniotomy. None of them had any history of intestinal or ano-rectal disorders. The children were divided into three groups according to age: Group one, 0-1 years of age (mean 4 months, range 2-10); group two, 5-7 years (mean 6); and group three, 10-12 years (mean 11). Each group consisted of 5 children.

METHODS

Equipment

Anal pressure was recorded at the level of the internal sphincter using a cuff of a Portex endotracheal tube no. 5.0. The tube was given a total length of 13-14 cm by cutting off the outer end. For children below one year of age, the cuff was tied off with a silk ligature to reduce its length to 1-1½ cm. The cuff was then filled with water and connected via a thin polyethylene tube to the recording equipment. The amount of water in the cuff was adjusted so that the cuff was expanded but the pressure inside it did not exceed zero.

Rectal distension was achieved with a latex balloon. When empty, this measured 1.5-2.0 cm. It was connected to a polyethylene tube about 50 cm long. Its internal diameter was 0 mm and its external 2.4 mm. This tube passed through the endotracheal tube and led to the recording equipment via a three-way stopcock through which air could be inflated into the balloon (Fig. 1).

The recording equipment comprised a pressure trans-

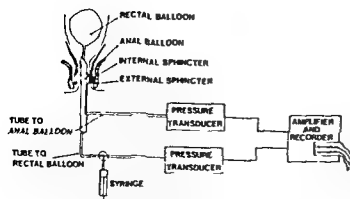


Fig 1 Diagrammatic representation of the method with an anteroposterior view of the rectal and anal balloons in situ

ducer (Statham P23) amplifier (Grass 7 P1) and recorder (Grass 7) connected separately to each line from the rectal balloon and the anal cuff. The same technique has been used earlier (6).

Procedure

The children below one year of age were lying on their backs with hips and knees in flexion; the older ones on their left side, also with a flexion in hips and knees. The rectal balloon was placed in position with the aid of some exploration cream and a swab. To ensure that the balloon lay unfolded, it was inflated with 5–10 ml of air, which was immediately evacuated. The anal balloon (i.e. the cuff of the endotracheal tube) was then placed just inside the anal verge, with the tube to the rectal balloon running through it (Fig 1). An assistant held this balloon in position during the examination.

Anal pressure was then recorded continuously. When the child was calm and the pressure stabilized, the rectal balloon was inflated with air: 5 and 10 ml in group one, 10 and 20 ml in group two, and 20 and 50 ml for the children in group three. Each inflation took less than 15 secs and the rectal balloon was kept filled for about 10–20 sec before it was evacuated. This was repeated several times.

Each child was examined twice: the first time awake and the second time during general anaesthesia. When examined awake the infants (i.e. group one) were premedicated with diazepam (Valium, Roche) 0.3 mg/kg body weight given orally one hour before the examination. The older children were not premedicated. The children were kept calm by the mother and in the case of the infants also with the aid of a feeding bottle or a dummy. When examined during anaesthesia the children were premedicated with morphine and atropine (Table 1) one hour before the examination. General anaesthesia was achieved with thionembutal sodium (Pentothal, Abbott) about 5 mg/kg body weight given intravenously and with inhalation of 80% nitrous oxide in oxygen.

Statistics

Standard statistical procedures were used. Means of two groups were compared with Student's paired *t* test (3). When comparing means of three groups, one way analysis

of variance was used (3). Data in the text and table are given as mean \pm S.E. (standard error of the mean).

RESULTS

All 15 children examined in this investigation were quiet, calm and cooperated well when awake. Conclusive recordings were thus obtained from all of them.

Anal pressure

Anal pressure at rest (Table 2) when the children were awake was significantly ($p < 0.05$) lower in group one (5.7 ± 0.75 kPa (43 ± 5.6 mmHg)) than in group two (9.5 ± 0.29 kPa (71 ± 2.2 mmHg)) and in group three (10.1 ± 0.68 kPa (76 ± 5.1 mmHg)). During general anaesthesia it decreased in all groups among infants, i.e. group one from 5.7 ± 0.75 kPa (43 ± 5.6 mmHg) to 4.7 ± 0.63 kPa (35 ± 4.7 mmHg) which is not fully significant. In group two it decreased from 9.5 ± 0.29 kPa (71 ± 2.2 mmHg) to 6.0 ± 0.44 kPa (45 ± 3.3 mmHg) and in group three from 10.1 ± 0.68 kPa (76 ± 5.1 mmHg) to 6.5 ± 0.48 kPa (49 ± 3.6 mmHg) which are both significant ($p < 0.05$) differences.

Table 1 Dose of morphine and atropine given as premedication before general anaesthesia

Body weight (kg)	Morphine (mg)	Atropine (mg)
3	0.1	0.05
4	0.1	0.05
5	0.15	0.1
6	0.2	0.1
7	0.3	0.2
8	0.4	0.2
9	0.5	0.2
10	0.6	0.2
12	1.0	0.2
14	1.5	0.3
16	2.2	0.3
18	2.6	0.3
20	3.8	0.3
22	4.5	0.5
24	5.3	0.5
26	6.0	0.5
28	6.8	0.5
30	7.5	0.5

Table 2 Anal pressure at rest (kPa) and relaxations (kPa) of the internal anal sphincter at different rectal volumes in the three groups of children when awake and under general anaesthesia (GA) respectively

	Anal pressure	Relaxations		
		10 ml	20 ml	50 ml
Group 1				
Awake	3.7±0.75	3.5±0.40	—	—
GA	4.7±0.63	2.7±0.45	—	—
Group 2				
Awake	9.5±0.79	3.1±0.40	3.5±0.79	—
GA	6.0±0.44	1.7±0.48	1.7±0.36	—
Group 3				
Awake	10.1±0.88	—	4.3±1.04	5.6±0.69
GA	6.5±0.48	—	2.9±0.73	3.5±0.56

denotes a significant difference ($p<0.05$) between the two conditions

Relaxation

Relaxations of the internal sphincter were recorded in all children both when awake and during general anaesthesia (Fig 2). The exact manner in which they were measured is indicated in Fig 2. i.e. the least anal pressure recorded during a relaxation was subtracted

from the resting anal pressure, the difference being regarded as the relaxation.

In general the relaxations were less pronounced during anaesthesia (Table 2). In group one when the rectal balloon was inflated with 10 ml they averaged 3.5 ± 0.40 kPa (26 ± 3.0 mmHg) when awake and 2.7 ± 0.45

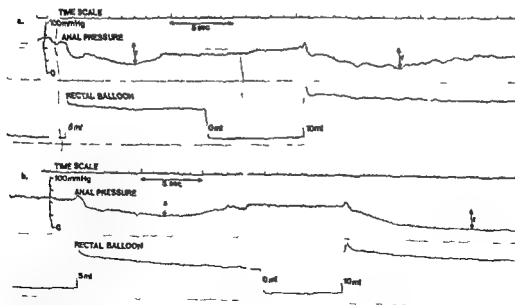


Fig 2a Recording of anal pressure during rectal distension in a 4 month-old boy when awake. In response to each rectal inflation there is a relaxation of the internal sphincter indicated with "r".

Fig 2b Recording of anal pressure during rectal distension in the same child but during general anaesthesia. Anal pressure at rest is slightly lower and the relaxations are slightly smaller.

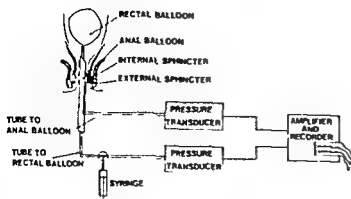


Fig 1 Diagrammatic representation of the method with an anteroposterior view of the rectal and anal balloons in situ

ducer (Stratham P23) amplifier (Grass 7 P1) and recorder (Grass 7) connected separately to each line from the rectal balloon and the anal cuff. The same technique has been used earlier (6).

Procedure

The children below one year of age were lying on their backs with hips and knees in flexion; the older ones on their left side, also with a flexion in hips and knees. The rectal balloon was placed in position with the aid of some exploration cream and a swab. To ensure that the balloon lay unfolded, it was inflated with 5–10 ml of air, which was immediately evacuated. The anal balloon (i.e. the cuff of the endotracheal tube) was then placed just inside the anal verge with the tube to the rectal balloon running through it (Fig. 1). An assistant held this balloon in position during the examination.

Anal pressure was then recorded continuously. When the child was calm and the pressure stabilized, the rectal balloon was inflated with air: 5 and 10 ml in group one, 10 and 20 ml in group two, and 20 and 50 ml for the children in group three. Each inflation took less than 0.5 sec and the rectal balloon was kept filled for about 10–20 sec before it was evacuated. This was repeated several times.

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Table 2 Anal pressure at rest (kPa) and relaxations (kPa) of the internal anal sphincter at different rectal volumes in the three groups of children when awake and under general anaesthesia (GA) respectively

	Anal pressure	Relaxations		
		10 ml	70 ml	50 ml
Group 1				
Awake	5.7±0.75	3.5±0.40	—	—
GA	4.7±0.63	2.7±0.45	—	—
Group 2				
Awake	9.5±0.79	3.1±0.40	3.5±0.79	—
GA	6.0±0.44	1.7±0.48	1.7±0.36	—
Group 3				
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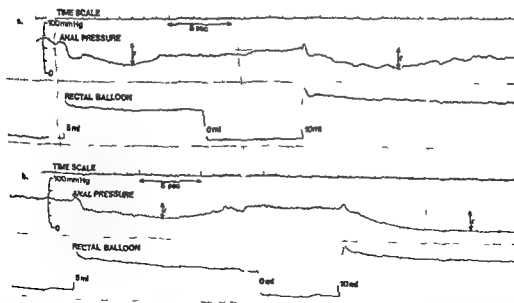


Fig 2a Recording of anal pressure during rectal distension in a 4 month-old boy when awake. In response to each rectal inflation there is a relaxation of the internal sphincter indicated with 'r'.

Fig 2b Recording of anal pressure during rectal distension in the same child but during general anaesthesia. Anal pressure at rest is slightly lower and the relaxations are slightly smaller.

kPa (20 ± 3.4 mmHg) during anaesthesia the difference being significant ($p < 0.05$). In group two the relaxations decreased from 3.1 ± 0.40 kPa (23 ± 3.0 mmHg) to 1.7 ± 0.48 kPa (13 ± 3.6 mmHg) with 10 ml in the rectal balloon and from 3.5 ± 0.79 kPa (26 ± 5.9 mmHg) to 1.7 ± 0.36 kPa (13 ± 2.7 mmHg) with 20 ml in the rectal balloon the first difference being significant ($p < 0.05$). Among the oldest children i.e. group three the relaxations decreased from 4.3 ± 1.04 kPa (32 ± 7.8 mmHg) to 2.9 ± 0.73 kPa (22 ± 5.5 mmHg) with 20 ml in the rectal balloon and from 5.6 ± 0.69 kPa (42 ± 5.2 mmHg) to 3.5 ± 0.56 kPa (26 ± 4.2 mmHg) ($p < 0.05$) with 50 ml in the rectal balloon.

Defecation

Four of the 5 infants in group one defecated during the examination when awake. This was preceded by a short period of very low anal pressure. Reflex defecation during the manometry did not occur in any of the older children nor did it occur in any child during general anaesthesia.

DISCUSSION

The anal canal is surrounded by the internal and external anal sphincters which both exhibit continuous tonic activity at rest. The pressure which can be recorded in the anal canal at rest is generated predominantly by the internal sphincter but to some extent by the external sphincter (4, 7). Consequently the decreased anal pressure observed in this investigation during general anaesthesia may theoretically be caused by a decreased activity in either sphincter. If it were due only to a lowered external sphincter activity however the recorded relaxation of the internal sphincter should not be reduced, as it was in this study. In fact a slight increase would be expected as the inflation reflex of the external sphincter is a reflex contraction in response to rectal distension (8) normally counteracts the internal

sphincter relaxation (7). It is therefore concluded that general anaesthesia with thiomebumal to some extent inhibits the tonic activity of the internal anal sphincter.

In response to rectal distension relaxations of the internal sphincter were recorded in all children both when awake and during general anaesthesia though the relaxations were significantly ($p < 0.05$) less pronounced during anaesthesia. This reduction is explained by the inhibited tonic activity of the internal sphincter at rest induced by thiomebumal as discussed above.

In other words the general anaesthesia inhibits the tonic activity of the internal anal sphincter and reduces the relaxation in response to rectal distension in healthy children. This rectosphincteric reflex is however still present and is not qualitatively affected by the general anaesthesia. The lack of internal sphincter relaxation in patients with Hirschsprung's disease is caused by the absence of ganglion cells and may therefore not be affected by general anaesthesia. Consequently anorectal manometry in the diagnosis of Hirschsprung's disease may be performed during general anaesthesia with thiomebumal if the child does not cooperate when awake.

Anal pressure at rest with the child awake was significantly ($p < 0.05$) lower in group one (5.7 ± 0.75 kPa 43 ± 5.6 mmHg) than in groups two (9.5 ± 0.29 kPa 71 ± 2.2 mmHg) and three (10.1 ± 0.68 kPa 76 ± 5.1 mmHg). The latter two groups did not differ essentially from adults in this respect (8.5 ± 0.47 kPa 64 ± 3.5 mmHg $n=10$) (7). This may indicate that anal sphincter activity is lower in infants than in older children and that it seems to increase and reach adult values after a few years of life i.e. when anal continence normally develops. However the absolute values may to some extent also have been influenced by the smaller size of the anal canal in infants but this possible source of error was partly compensated for by the shorter anal cuff that was used in this group.

The diagnosis of Hirschsprung's disease can

usually be established with the aid of barium enema examination and conventional rectal biopsy. The interpretation of X rays findings may however be difficult in some patients and rectal biopsy is not without complications and mortality (5). Consequently ano rectal manometry is a valuable complement in the diagnosis as several studies show that its reliability in this context is high (1, 6, 10, 14, 15, 16). Conclusive recordings require a quiet, calm child which is mostly the case. In the few instances however when the patient does not cooperate it is suggested on the basis of the present investigation that ano-rectal manometry may be performed with advantage during general anaesthesia.

REFERENCES

- 1 Aaronson I & Nixon H H. A clinical evaluation of ano-rectal pressure studies in the diagnosis of Hirschsprung's disease. *Gut* 13: 138 1972.
- 2 Denny Brown D & Robertson E G. An investigation of the nervous control of defecation. *Brain* 58: 246 1935.
- 3 Dixon W H & Massey F J. *Introduction to Statistical Analysis*. McGraw Hill, New York 1957.
- 4 Duthie H L & Watts J M. Contribution of the external anal sphincter to the pressure zone in the anal canal. *Gut* 6: 64 1965.
- 5 Fraser G C & Wilkinson A W. Neonatal Hirschsprung's disease. *Br Med J* in 7 1967.
- 6 Frenckner H. Ano-rectal manometry in the diagnosis of Hirschsprung's disease in infants. *Acta Paediatr Scand* 67: 187 1978.

- 7 Frenckner H & von Euler Chr. Influence of pudendal block on the function of the anal sphincters. *Gut* 16: 487 1975.
- 8 Gaston E A. The physiology of fecal continence. *Surg Gynecol Obstet* 87: 280 1948.
- 9 Gowers W E. The autonomic action of the sphincter ani. *Proc R Soc Lond* 26: 77 1877.
- 10 Holtschneider A M. Moderne Funktionsdiagnostik anorektaler Erkrankungen im Kindesalter. *Munch Med Wochenschr* 116: 1179 1974.
- 11 Lawson J H N & Nixon H H. Anal canal pressure in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 2: 544 1967.
- 12 Schnauffer L, Talbert J L, Haller J A, Reid N C R W, Tobon F & Schuster M M. Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood. *J Pediatr Surg* 2: 538 1967.
- 13 Schuster M M, Hendrix T R & Mendeloff A J. The internal anal sphincter response: Manometric studies on its normal physiology, neural pathways and alterations in bowel disorders. *J Clin Invest* 42: 196 1963.
- 14 Tobon F, Reid N C R W, Talbert J L & Schuster M M. Nonsurgical test for the diagnosis of Hirschsprung's disease. *N Engl J Med* 278: 188 1968.
- 15 Varma K K & Stephens D. Neuromuscular reflexes of rectal continence. *Aust NZ J Surg* 41: 763 1971.
- 16 Verder H, Johansen K S & Engbaek K. Anal tonometry: A diagnostic help in Hirschsprung's disease. *Acta Paediatr Scand* 62: 59 1973.

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ASSESSMENT OF LUNG FUNCTION ON HEALTHY CHILDREN USING AN ELECTRONIC SPIROMETER AND AN AIR FLOWMETER BEFORE AND AFTER INHALATION OF AN ADRENERGIC RECEPTOR STIMULANT

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ABSTRACT Dalén G and Kjellman B (Department of Paediatrics, Karnsjukhuset Skövde, Sweden). Assessment of lung function on healthy children using an electronic spirometer and an Air Flowmeter before and after inhalation of an adrenergic receptor stimulant. *Acta Paediatr Scand* 68: 103-108, 1979. — VC measured with a Monaghan electronic spirometer equipped with a backflow valve is significantly lower (about 4%) than when measured with the same spirometer without such a valve. The measurements of FEV_{1.0} were not influenced by the valve. 73 healthy children were investigated with the Monaghan spirometer equipped with the backflow valve and normal reference data were established. The results were very similar to those obtained in an investigation of healthy children with the same spirometer about one year earlier. Reference data on children for a simple flow meter, Airflometer (Glaxo Ltd), are given. The data correlated very highly to the FEV_{1.0} values obtained by the Monaghan spirometer. After inhalation of salbutamol healthy children had a small and significant increase of FEV_{1.0} and of the Airflometer value but not of VC. The deviations of the differences were small. A 6% increase of VC and 10% increase of FEV_{1.0} were taken as normal upper limits after inhalation of salbutamol. Corresponding increase of the Airflometer values was 15 arbitrary units for children with body heights 116-145 cm and 21 units for children with body heights 146-175 cm.

KEY WORDS Children, spirometry, electronic spirometer, Airflometer, salbutamol, inhalation.

A desirable increase of pulmonary function tests on children with bronchial or lung diseases requires equipment with such characteristics as simple operation and fast delivery of data. The equipment must also be reliable and it is important that evaluation of it is done with regard to precision and accuracy and it is also important to establish reference data on healthy subjects.

In a previous study (3) on healthy children we compared two electronic spirometers (Spirotron Dräger and Monaghan M 403) and one wedge bellows spirometer (Vitalograph) with a water spirometer. The Monaghan spirometer was used without the backflow valve which may have presented the risk of getting erroneously high values of vital capacity

(VC). The comparison showed encouraging results but further evaluation of the spirometers was suggested.

A new simple flow meter, Airflometer (AFM, Glaxo Ltd), has shown good performance in studies on adults and in phantom investigations (1, 6, 8). A high correlation of the AFM values was found expressed in arbitrary units (AFM units) to the forced expiratory volume in one second (FEV_{1.0}) obtained in spirometry. These results have encouraged us to the present evaluation of the AFM on children.

The aims of the present study are:

1. to compare the values given by a Monaghan spirometer with its backflow valve and without it.

Table 2 *Vital capacity (VC) forced expiratory volume in one second (FEV_{1.0}) in relation to body height in metres (H)*

There are given data from Monaghan spirometry with a backflow valve (M1) i.e. the present investigation, data of Monaghan spirometry without a valve (M2) and data of Bernstein spirometry (B). The data of M2 and B were obtained in a previous investigation (3). Standard deviations (SD) are expressed in litres and in percentages of the mean VC or mean FEV.

Lung function	Spiro-meter	n	Equation of the regression lines	S D		Coefficient of correlation
				l	%	
VC boys	M 1	34	$0.787 \times H^3 + 0.077$	0.33	12	0.98
	M 2	31	$0.833 \times H - 0.001$	0.78	11	0.98
	B	31	$0.781 \times H^3 + 0.137$	0.27	11	0.98
VC girls	M 1	39	$0.759 \times H^3 + 0.000$	0.74	10	0.98
	M 2	30	$0.976 \times H^3 - 0.464$	0.27	11	0.97
	B	30	$0.768 \times H - 0.039$	0.74	10	0.96
FEV boys	M 1	34	$0.675 \times H^3 + 0.244$	0.26	11	0.98
	M 2	31	$0.657 \times H^3 + 0.159$	0.71	10	0.98
	B	31	$0.671 \times H^3 + 0.100$	0.21	10	0.98
FEV girls	M 1	39	$0.700 \times H - 0.058$	0.72	10	0.98
	M 2	30	$0.710 \times H^3 - 0.108$	0.18	9	0.97
	B	30	$0.690 \times H^3 - 0.039$	0.19	9	0.97

without a backflow valve showed significantly higher mean VC in spirometry without a valve (Table 1). No systematic difference was found for FEV_{1.0}. Table 2 shows the equations of the regression lines of VC and FEV_{1.0} with respect to body height (raised to the third power) for the 73 healthy children. The S D around the lines and the correlation coefficients are also given. The same data of our previous study about one year ago are presented for comparison.

To illustrate the similarity of the reference data obtained with the Monaghan spirometer in the two studies, the values are given for three body heights in Table 3. For comparison the reference data of four Swedish studies using water spirometers are also shown. The Table shows that the differences between the water spirometers are larger than the differences between the two studies of the Monaghan spirometer.

Table 4 shows the equations of the regres-

Table 3 *Vital capacity (VC) and forced expiratory volume in one second (FEV_{1.0}) in healthy children*

The results of Monaghan spirometry with a backflow valve and without such a valve are given for the body heights 130 cm, 145 cm and 160 cm. For comparison corresponding values of four Swedish studies on healthy children with water spirometers are also given.

Spirometer	VC (l)						FEV _{1.0} (l)					
	130 cm		145 cm		160 cm		130 cm		145 cm		160 cm	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Monaghan valve	1.80	1.67	2.46	2.31	3.78	3.10	1.67	1.48	2.15	2.08	2.80	2.81
Monaghan no valve (3)	1.83	1.57	2.54	2.36	3.41	3.33	1.60	1.45	2.15	2.06	2.83	2.80
Bernstein (3)	1.85	1.65	2.51	2.30	3.33	3.11	1.57	1.46	2.14	2.05	2.85	2.77
Bernstein ()	1.96	00	2.78	2.63	3.77	3.40	1.71	1.72	2.37	2.30	3.19	3.07
Bernstein (5)*		1.65		2.73		2.93		1.47		1.89		2.44
Bernstein (7)*		1.65		2.6		3.00		1.46		1.97		2.63

This study gives the results at BTPS for boys and girls, why the values given in this table are recalculated to ATPS.

of VC probably depend upon the backflow valve in the way discussed above. Other investigations of healthy Swedish children with water spirometers have however shown larger differences in results with one and the same type of spirometer i.e. water spirometers than those obtained by us on two different occasions with the Monaghan spirometer (2, 3, 5, 7).

In our study a very high correlation ($r = 0.99$) was found between $FEV_{1.0}$ of the Monaghan spirometer and the AFM units which agrees with findings on children and adults (1 & 11). Previous phantom studies have also disclosed good precision in this simple instrument (1, 8). The correlation to $FEV_{1.0}$ for the AFM should be better than for the Wright peak flow meter because not only the flow rate but also the time of flow influences the result. To get measurable values of the AFM the subject must be capable of an exhalation corresponding to a $FEV_{1.0}$ of about one litre which is a drawback when investigating small children. All of our children did however cooperate very satisfactorily in the AFM maneuver and all of them gave measurable results. The correlation of AFM to body height is as high as that of $FEV_{1.0}$ whereas the SD values around the lines are larger than those of $FEV_{1.0}$ which agrees with previous findings on adults (1). Previous studies have shown a high reproducibility for one and the same AFM whereas the variations sometimes are considerable among different apparatuses (1, 8, 11).

In proper spirometric investigations of subjects with bronchial diseases the tests should also be done after administration of a bronchodilating drug. In Sweden inhalations of salbutamol solution with compressor nebulizers are often used as the first treatment of acute asthma and also in testing the bronchial reaction for diagnostic purposes. The bronchial response of healthy children was not previously evaluated but it has often been said that an increase of $FEV_{1.0}$ of about 10% can be considered normal. Souhrada & Buckley

(9) state that significance is established only when the change in the spirometer values is 20% or greater. In the study by Svenonius et al. (10) 10 healthy children were investigated after exercise and inhalations of salbutamol. There were no changes of mean VC or mean $FEV_{1.0}$. In our study of 62 healthy children there was no systematic change in mean VC after inhalation of salbutamol (Table 5). Furthermore the deviations of the VC differences are small and not greater than those found in an ordinary duplicate investigation with a water spirometer (4, 5). There was however a systematic change of $FEV_{1.0}$. Despite the disturbing intermediate moment of inhalation the deviations of the $FEV_{1.0}$ differences were not greater than the deviations found in an ordinary duplicate investigation with a water spirometer (5).

We have chosen a 6% increase of VC and 10% increase of $FEV_{1.0}$ after administrations of salbutamol as normal upper limits which correspond to a value just above $M+2$ SD (Table 5).

Calculation of the change after inhalation of salbutamol in per cent is not possible for the AFM and the upper normal limits of change must be given in absolute AFM units. An analysis of the change in relation to body height and original AFM values (Table 5) resulted in two limits: an increase of 15 AFM units for the heights 116–145 cm and 21 AFM units for the heights 146–175 cm.

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REFERENCES

1. Arborelius M & Christensson P. Airlometer—en enkel apparat för lungfunktionstest. *Läkartidn* 75: 38, 1978.
2. Bjure J. Spirometric studies in normal subjects. IV. Ventilatory capacities in healthy children 7–17 years of age. *Acta Paediatr Scand* 52: 232, 1963.
3. Dalen O & Kjellman M. Evaluation of three spirometers on healthy children. *Acta Paediatr Scand* in press.

Table 4 The relations of the Airflometer values (AFM) to body height (H) and forced expiratory volume in one second (FEV_{1.0}) to AFM in healthy children

The equations of linear regression, the standard deviations (S D) and the coefficients of correlation (r) are given. AFM is expressed in arbitrary units. FEV_{1.0} in litres and H in metres

Regression	Equation	S D	r
AFM to H			
Boys	$AFM = 45.78 \times H^2 - 38.88$	25	0.97
Girls	$AFM = 54.33 \times H^2 - 71.28$	17	0.98
FEV _{1.0} to AFM			
Boys	$FEV_{1.0} = AFM \times 0.01308 + 0.8418$	0.15	0.99
Girls	$FEV_{1.0} = AFM \times 0.01272 + 0.8768$	0.13	0.99

sion lines of the AFM with respect to the body heights of the 73 healthy children. The high correlation of the AFM units to the FEV_{1.0} on the 73 healthy children is evident from Table 4 and Fig. 1.

Changes of VC, FEV_{1.0} and AFM units on healthy children after inhalation of salbutamol are shown in Table 5. The increase of mean VC was not significant whereas the increases of mean FEV_{1.0} and the mean AFM units are systematic. For the two first groups of body heights 116–130 cm and 131–145 cm the changes of the AFM units are very similar and less than the change for the body heights 146–175 cm.

DISCUSSION

According to the manufacturer the results should be the same whether the Monaghan spirometer is used with or without a backflow valve. Our results show that this is true of FEV_{1.0} but not of VC. A mean difference of 0.14 l (Table 1) implies 4% of the mean VC value. This systematic difference may depend upon the fact that some children begin to inhale before the removal of the mouthpiece after the FVC maneuver. If the backflow valve is not used the inhalation causes a change of temperature recorded by the thermistor of the spirometer which gives erroneously high VC values. A substantial delay in the removal of the mouthpiece is probably observed by the investigator whereas a small delay can be overlooked. This implies that the

values obtained with the spirometer equipped with a valve should be the more correct ones. For this reason we prefer to use the spirometer equipped with a valve.

Our new reference data for FEV_{1.0} of the Monaghan spirometer equipped with a backflow valve should thus be very similar to the data obtained in the investigation one year ago which is also the case (Table 2). The very close agreement of the FEV_{1.0} values does credit to the spirometer. The differences

Table 5 Paired differences of vital capacity (VC) forced expiratory volume in one second (FEV_{1.0}) and Airflometer values (AFM) before and after inhalation of salbutamol (0.15 mg per kg body weight)

For VC and FEV_{1.0} the differences are expressed in per centages of the values before inhalation and within brackets in absolute values (l). For AFM the differences are given in absolute values in arbitrary units and the results are divided in four groups according to body height.

Lung function	M	S D	M+2 S D
VC			
Boys	+1.1 (0.03)	2.2 (0.08)	5.6
Girls	+0.2 (0.01)	1.6 (0.06)	3.4
FEV _{1.0}			
Boys	+3.6 (0.09)	2.3 (0.08)	8.1
Girls	+3.5 (0.07)**	2.7 (0.07)	8.9
AFM body height (cm)			
116–130	+5.8**	4.6	15.0
131–145	+4.8*	5.0	14.8
146–160	+9.6*	5.9	21.4
161–175	+8.9*	5.7	20.3

* = not significant

** = < 0.01

of VC probably depend upon the backflow valve in the way discussed above. Other investigations of healthy Swedish children with water spirometers have however shown larger differences in results with one and the same type of spirometer i.e. water spirometers than those obtained by us on two different occasions with the Monaghan spirometer (2, 3, 5, 7).

In our study a very high correlation ($r = 0.99$) was found between $FEV_{1.0}$ of the Monaghan spirometer and the AFM units which agrees with findings on children and adults (1, 6, 11). Previous phantom studies have also disclosed good precision in this simple instrument (1, 8). The correlation to $FEV_{1.0}$ for the AFM should be better than for the Wright peak flow meter because not only the flow rate but also the time of flow influences the result. To get measurable values of the AFM the subject must be capable of an exhalation corresponding to a $FEV_{1.0}$ of about one litre which is a drawback when investigating small children. All of our children did however cooperate very satisfactorily in the AFM maneuver and all of them gave measurable results. The correlation of AFM to body height is as high as that of $FEV_{1.0}$ whereas the SD values around the lines are larger than those of $FEV_{1.0}$ which agrees with previous findings on adults (1). Previous studies have shown a high reproducibility for one and the same AFM whereas the variations sometimes are considerable among different apparatuses (1, 8, 11).

In proper spirometric investigations of subjects with bronchial diseases the tests should also be done after administration of a bronchodilating drug. In Sweden inhalations of salbutamol solution with compressor nebulizers are often used as the first treatment of acute asthma and also in testing the bronchial reaction for diagnostic purposes. The bronchial response of healthy children was not previously evaluated but it has often been said that an increase of $FEV_{1.0}$ of about 10% can be considered normal. Souhrada & Buckley

(9) state that significance is established only when the change in the spirometer values is 20% or greater. In the study by Svenonius et al. (10) 10 healthy children were investigated after exercise and inhalations of salbutamol. There were no changes of mean VC or mean $FEV_{1.0}$. In our study of 62 healthy children there was no systematic change in mean VC after inhalation of salbutamol (Table 5). Furthermore the deviations of the VC differences are small and not greater than those found in an ordinary duplicate investigation with a water spirometer (4, 5). There was however a systematic change of $FEV_{1.0}$. Despite the disturbing intermediate moment of inhalation the deviations of the $FEV_{1.0}$ differences were not greater than the deviations found in an ordinary duplicate investigation with a water spirometer (5).

We have chosen a 6% increase of VC and 10% increase of $FEV_{1.0}$ after administrations of salbutamol as normal upper limits which correspond to a value just above $M+2$ SD (Table 5).

Calculation of the change after inhalation of salbutamol in per cent is not possible for the AFM and the upper normal limits of change must be given in absolute AFM units. An analysis of the change in relation to body height and original AFM values (Table 5) resulted in two limits: an increase of 15 AFM units for the heights 116–145 cm and 21 AFM units for the heights 146–175 cm.

ACKNOWLEDGEMENT

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REFERENCES

1. Arborelius M & Christensson P. Airflowmeter—enkel apparat för lungfunktionstest. *Läkartidn* 75: 38, 1978.
2. Bjure J. Spirometric studies in normal subjects. IV. Ventilatory capacities in healthy children 7–17 years of age. *Acta Paediatr Scand* 52: 237, 1963.
3. Dalen G & Kjellman B. Evaluation of three spirometers on healthy children. *Acta Paediatr Scand* in press.

Table 4 The relations of the Airflowmeter values (AFM) to body height (H) and forced expiratory volume in one second (FEV_{1.0}) to AFM in healthy children

The equations of linear regression, the standard deviations (S D) and the coefficients of correlation (r) are given. AFM is expressed in arbitrary units, FEV_{1.0} in litres and H in metres

Regression	Equation	S D	r
AFM to H			
Boys	$AFM = 45.78 \times H^2 - 38.88$	25	0.97
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Table 5 Paired differences of vital capacity (VC), forced expiratory volume in one second (FEV_{1.0}) and Airflowmeter values (AFM) before and after inhalation of salbutamol (0.15 mg per kg body weight)

For VC and FEV_{1.0} the differences are expressed in per centages of the values before inhalation and within brackets in absolute values (l). For AFM the differences are given in absolute values, i.e. arbitrary units and the results are divided in four groups according to body height.

Lung function	M	S D	M+2 S D
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161–175	+8.9*	5.7	20.3

* = not significant ** = $p < 0.01$

REDUCED SERUM 1,25 (OH)₂ VITAMIN D₃ LEVELS IN PREDNISONE TREATED ADOLESCENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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ABSTRACT O'Regan S, Chesney R W, Hamstra A, Eisman J A, O'Gorman A M and DeLuca H F (Departments of Nephrology and Radiology, McGill University, Montreal Children's Hospital, Montreal, Canada, Research Institute, Montreal, Canada and the Departments of Pediatrics and Biochemistry, University of Wisconsin, Madison, Wisc, USA). Reduced serum 1,25-(OH)₂ vitamin D levels in prednisone treated adolescents with systemic lupus erythematosus. *Acta Paediatr Scand* 68: 109, 1979. —The serum levels of 1,25-(OH)₂ vitamin D₃ were assayed in samples from 12 adolescent patients with SLE. Subnormal levels were observed in 7 of these 12 patients. Low levels of the metabolically active polar metabolite of vitamin D may contribute to the development of osteopenia observed in this disease. The cumulative effects of the osteoporotic and anti-vitamin D effects of long term steroid therapy in children with SLE may require the cautious administration of supplemental vitamin D.

KEY WORDS 1,25 (OH)₂ D₃, steroids, SLE

Recent studies suggest that avascular necrosis of bone may be a common complication of systemic lupus erythematosus (SLE) (1-7). These bone lesions, usually adjacent to joint surfaces, may cause severe pain or incapacitating structural deformities. Although steroids have been considered to be a contributing factor, the diffuse vasculitis of SLE has also been suggested as a possible cause (1), particularly since osteonecrosis has also occurred in the absence of steroid therapy (3-10). Thus steroid therapy has obscured the distinction between the role of the disease itself and that of the treatment in the pathogenesis of bone disease in SLE.

To determine if derangements in vitamin D₃ metabolism — a major factor in normal bone deposition and reabsorption — might exist and thus possibly contribute to the development of bone lesions in SLE, we have assayed the serum levels of 1,25 (OH)₂ D₃, a major active

polar metabolite of vitamin D₃ in 12 children with SLE of long duration and thus at risk for developing bone lesions. This study also afforded the opportunity to determine if chronic steroid therapy might be associated with altered 1,25 (OH)₂ vitamin D₃ metabolism as previous reports have indicated that both normal (6) and decreased (8) serum 25 (OH) D₃ levels may be associated with chronic steroid ingestion.

MATERIALS AND METHODS

Serum samples of 17 children with SLE were assayed for 1,25-(OH)₂ D₃. The diagnosis of SLE was confirmed by the presence of laboratory positive LE cell preparations, elevated antinuclear antibody (ANA) titers, low serum C levels and clinical features consistent with the diagnosis. All had lupus nephritis confirmed by light electron and immunofluorescence microscope examination of renal biopsy material. All had been or were undergoing treatment with a combination of prednisone and azathioprine. All available radiologic examinations were re-examined.

- 4 Engström I Karlberg P & Kriepelien S Respiratory studies in children I Lung volumes in healthy children 6-14 years of age *Acta Paediatr Scand* 46 277 1956
- 5 Engström I Karlberg P & Swarts C L Respiratory studies in children IX Relationships between mechanical properties of the lungs lung volumes and ventilatory capacity in healthy children 7-15 year of age *Acta Paediatr Scand* 51 68 1962
- 6 Friedman M & Walker S Assessment of lung function using an air flowmeter *Lancet* i 310 1975
- 7 Kjellman B Ventilatory efficiency capacity and lung volumes in healthy children *Scand J Clin Lab Invest* 23 19 1969
- 8 Ringqvist I Sixt R Bake B & Oshay H Validity of the Airflometer A simple device for assessment of forced expiration *Scand J Resp Dis* 58 265 1977
- 9 Souhrada J F & Buckley J M Pulmonary function testing in asthmatic children *Ped Clin North Amer* 23 249 1976
- 10 Svenonius E Lecerof H Lilja B & Arborelius M Jr The volume of trapped gas A new and sensitive test for the detection of exercise induced bronchospasm in children *Acta Paediatr Scand* in press
- 11 Thompson P Friedman M & Walker S The Airflometer—A new device for assessing changes in respiratory function *Practitioner* 219 3 1977

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min D₃ therapy at 0.4 µg per day restored normal intestinal calcium transport (5). It appeared that daily prednisone therapy was necessary to produce decreased 25 (OH) vitamin D₃ levels though Hahn et al. (6) have recently reported normal serum levels of 25 (OH) D₃ levels in patients on chronic corticosteroid therapy. However, in our adolescent patients prednisone therapy was associated with reduced 1 25 (OH)₂ vitamin D₃ levels. Though serum urea nitrogen and creatinine levels were normal, this does not rule out the possibility that tubular function was inadequate for 1α hydroxylation although, as mentioned above, steroid therapy may alter 1α 25 (OH) D₃ metabolism (2). Dietary availability of vitamin D was >250 units/daily in all these patients; hence, inadequate intake was unlikely. Flordrachitic changes were not evident radiologically in any of the patients. Low calcium levels were evident in only two cases and these patients also had low 1-25 (OH) D₃ levels.

The observation that 7 of 12 children with SLE had low levels of 1 25 (OH) D₃ possibly related to chronic steroid therapy of vitamin D₃ suggests that routine dietary intake may be inadequate for these patients. The cumulative effects of the osteoporotic and anti-vitamin D effects of long-term steroid therapy in children with SLE may require the cautious administration of supplemental vitamin D, as has been used in other patients with exogenous hypercortisolemia (5, 8).

REFERENCES

1. Bergstein J M, Wiens C, Fish A J, Vernier R L & Michalek A. Avascular necrosis of bone in systemic lupus erythematosus. *J Pediatr* 85: 31, 1974.

2. Carre M, Ayigbede P, Miravet L & Rasmussen H. The effect of prednisone upon the metabolism and action of 25 Hydroxy and 1 25 D hydroxy vitamin D₃. *Proc Natl Acad Sci* 71: 2996, 1974.
3. Dubois E L & Cozen L. Avascular (aseptic) bone necrosis associated with systemic lupus erythematosus. *JAMA* 174: 966, 1960.
4. Eisman J A, Hauser A J, Kream B E & DeLuca H F. 1 25 D Hydroxy vitamin D in biological fluids: a simplified and sensitive assay. *Science* 193: 1071, 1976.
5. Hahn T J & Hahn B H. Osteopenia in patients with rheumatic diseases: principles of diagnosis and therapy. *Semin Arthritis Rheum* 6: 165, 1976.
6. Hahn T J, Halstead L R & Haddad J G. Serum 25 Hydroxy vitamin D concentration in patients receiving chronic corticosteroid therapy. *J Lab Clin Med* 90: 399, 1977.
7. Hurley B M, Steinberg R M, Patinkin H & Drummond K N. Avascular necrosis of the femoral head in childhood systemic lupus erythematosus. *Can Med Assoc J* 111: 79, 1974.
8. Klein R G, Arnaud S B, Gallagher J C, DeLuca H F & Riggs B L. Intestinal calcium absorption in exogenous hypercortisolemia: Role of 25 hydroxy vitamin D and corticosteroid dose. *J Clin Invest* 60: 253, 1977.
9. Lukert B P, Stanbury S W & Mawer E B. Vitamin D and intestinal transport of calcium: effects of prednisone. *Endocrinology* 93: 718, 1973.
10. Siemsen J K, Brook J & Meister L. Lupus erythematosus and avascular bone necrosis: a clinical study of three cases and review of the literature. *Arthritis Rheum* 5: 497, 1967.
11. Sutton M D. Aseptic necrosis of bone: a complication of corticosteroid therapy. *Drug Ind Dis* 3: 171, 1968.

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Table 1 Serum 1 25 (OH)₂ D₃ levels in SLE patients

Patient no	Duration of disease (years)	Radiologic assessment	Serum Ca Phos Alk Phos	Serum 1 25 (OH) D ₃ pg/ml (normal 40.8 ± 2.6 pg/ml)
1	1	Mild osteopenia	Normal	12
2	5	Normal	Normal	17
3	7	Mild osteopenia	Normal	30
4	8	Mild osteopenia	Normal	115 (on vit D ₃)
5	4	Mild osteopenia	Normal	15
6	4	Mild osteopenia	Normal	32
7	5	Normal	Serum Cal	25
8	1	Mild osteopenia	Normal	11
9	1	Mild osteopenia	Normal	62 (on vit D ₃)
10	2	Mild osteopenia	Normal	59
11	5	Normal	Normal	82
12	3	Mild osteopenia	Normal	48
			Serum Cal	4
				0
				42
				12

for evidence of bone lesions. The serum C₃ level, ANA and parameters of renal function were also assessed. Serum Ca, phosphate and alkaline phosphatase levels were also measured using a Technicon autoanalyzer. 1 25-(OH)₂ D₃ levels were assayed by a modification of the method of Eisman et al. (4). This assay is specific for 1 25 (OH)₂ D₃. Values obtained were compared with values obtained in 56 normal children of ages 8–18 years.

RESULTS

Duration of disease in these patients varied from one to eight years. Patient ages varied from 12–19 years. There were 8 females and 4 males. Normal values for 1 25 (OH)₂ vitamin D₃ in healthy children ages 8–18 was 40.8 ± 2.6 pg/ml (mean ± 1 S.E.) (range 19–111 pg/ml). Seven of the 12 patients had subnormal levels of 1 25 (OH)₂ D₃ (Table 1). On later assay 2 of the 7 had normal levels.

All patients had normal serum creatinine and BUN concentrations. Liver function studies (SGOT, SGPT, LDH) available on 6 patients were normal. Eleven patients had received or were receiving prednisone 50–80 mg every other day and azathioprine therapy 50 mg daily. One patient was receiving prednisone 60 mg/day. Serum calcium, phosphate and alkaline phosphatase were normal in all but 2 patients. Radiographic studies available in all patients did not demonstrate any major bone

lesions or rickets changes, though osteopenia was prominent on many of the examinations. Dietary history revealed an intake of >250 units vitamin D₃ per day in each patient. Patients are told to avoid sunlight and to use various sunscreen agents when exposure was anticipated.

DISCUSSION

Previous studies of children and adults with SLE indicate this group of patients is at risk for developing bone lesions. Corticosteroid administration in SLE and other diseases has been associated with osteonecrosis (11) and osteoporosis. This may in part be related to the inhibition of the intestinal transport of calcium by steroids (9). Steroids have been demonstrated to alter 1 25 (OH) D₃ metabolism in the rat leading to the formation of more polar biologically inactive metabolites that may result in decreased intestinal calcium transport (2). Recently it has been demonstrated that serum 25 (OH) vitamin D₃ levels are reduced in corticosteroid treated adult patients with a variety of collagen vascular diseases and rheumatoid arthritis (8). The reduction in gut calcium transport in these patients appeared to be related to the lower serum 25 (OH) vitamin D₃ levels and treatment with 1 25 (OH)₂ vita

min D₃ therapy at 0.4 µg per day restored normal intestinal calcium transport (5). It appeared that daily prednisone therapy was necessary to produce decreased 25 (OH) vitamin D₃ levels though Hahn et al. (6) have recently reported normal serum levels of 25 (OH) D₃ levels in patients on chronic corticosteroid therapy. However in our adolescent patients prednisone therapy was associated with reduced 1 25 (OH)₂ vitamin D₃ levels. Though serum urea nitrogen and creatinine levels were normal this does not rule out the possibility that tubular function was inadequate for 1α Hydroxylation although as mentioned above steroid therapy may alter 1α 25 (OH)-D₃ metabolism (2). Dietary availability of vitamin D was >250 units/daily in all these patients hence inadequate intake was unlikely. Flord rachitic changes were not evident radiologically in any of the patients. Low calcium levels were evident in only two cases and these patients also had low 1-25 (OH) D₃ levels.

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REFERENCES

- Bergstein J M, Wiens C, Fish A J, Vermer R L & Michael A. Avascular necrosis of bone in systemic lupus erythematosus. *J Pediatr* 85: 31, 1974.

- Carre M, Ayigbede P, Miravet L & Rasmussen H. The effect of prednisone upon the metabolism and action of 25 Hydroxy and 1 25 D hydroxy vitamin D₃. *Proc Natl Acad Sci* 71: 2996, 1974.
- Dubois E L & Cozen L. Avascular (aseptic) bone necrosis associated with systemic lupus erythematosus. *JAMA* 174: 966, 1960.
- Eisman J A, Hausta A J, Kream B E & DeLuca H F. 1 25 D Hydroxy vitamin D₃ in biological fluids: a simplified and sensitive assay. *Science* 193: 1071, 1976.
- Hahn T J & Hahn B H. Osteopenia in patients with rheumatic diseases: principles of diagnosis and therapy. *Semin Arthritis Rheum* 6: 165, 1976.
- Hahn T J, Halstead L R & Haddad J G. Serum 25 Hydroxy vitamin D concentration in patients receiving chronic corticosteroid therapy. *J Lab Clin Med* 90: 399, 1977.
- Hurley B M, Steinberg H M, Patinkin H & Drummond K N. Avascular necrosis of the femoral head in childhood systemic lupus erythematosus. *Can Med Assoc J* 111: 79, 1974.
- Klem M G, Arnaud S M, Gallagher J C, DeLuca H F & Riggs B L. Intestinal calcium absorption in exogenous hypercortisolemia: Role of 25 hydroxy vitamin D and corticosteroid dose. *J Clin Invest* 60: 253, 1977.
- Lukert B P, Stanbury S W & Mawer E B. Vitamin D and intestinal transport of calcium: effects of prednisone. *Endocrinology* 93: 718, 1973.
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EFFECT OF INTRAVENOUS HYDROCORTISONE ADMINISTRATION ON GLUCOSE HOMEOSTASIS IN SMALL FOR GESTATIONAL AGE INFANTS

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ABSTRACT Sann L, Ruitton A, Mathieu M and Lasne Y (Department of Neonatology, INSERM U34 and Laboratory of Endocrinology, Hôpital Debrousse, Lyon, France). Effect of intravenous hydrocortisone administration in small for gestational age infants. *Acta Paediatr Scand* 68 113-118 1979. The effects of 1% hydrocortisone (H) (10 mg/kg) on glucose homeostasis were evaluated at 25 \pm 85 hours of age in 14 infants who were small for gestational age (SGA) in comparison with 17 control SGA infants. Three hours after H administration, higher levels of plasma glucose than in controls were detected (mean \pm S.E.M.) 4.78 ± 0.2 vs 2.88 ± 0.2 mmol/l ($p < 0.01$), while lower levels were found for blood pyruvate (38 ± 7 vs 89 ± 11 μ mol/l- $p < 0.01$), plasma insulin (6.4 ± 0.5 vs 12 ± 0.8 μ U/ml- $p < 0.05$) and plasma glucagon (62.25 ± 6.6 vs 81.6 ± 6 pmol/l- $p < 0.05$). Three hours after H administration, 1% injection of L-alanine (150 mg/kg) produced a significant rise over baseline of plasma glucose concentration from 4.78 ± 0.2 to 5.94 ± 0.2 mmol/l \pm 50 min ($p < 0.05$), whereas no significant change was observed in controls. There was no significant change in plasma glucagon and insulin concentrations after L-alanine injection in either group. These results show that in SGA infants primed with H, the rise of plasma glucose concentration after L-alanine administration is observed with low plasma insulin levels and without stimulation of glucagon secretion. They suggest that H induced a reduced peripheral utilization of glucose by lowering the plasma levels of insulin and a production of glucose from alanine through gluconeogenesis.

KEY WORDS Infants, glucose homeostasis, hydrocortisone, plasma insulin, alanine, gluconeogenesis.

Small for gestational age (SGA) infants are at risk from hypoglycemia (2, 12). This predisposition to hypoglycemia is attributed to decreased hepatic glycogen stores (3, 24) and/or inadequate gluconeogenesis (11). A major treatment for neonatal hypoglycemia is provided by glucocorticoids like hydrocortisone (H) (2, 24). Hyperglycemia induced by corticoids is due to reduced peripheral utilization of glucose (17), increased secretion and effect of glucagon (27) and enhanced gluconeogenesis (5, 23). In newborn infants, H has been shown to reduce peripheral glucose utilization (9) and to increase the effect of exogenous glucagon (18). But the other mechanisms have not been investigated.

The present work was designed to study

in SGA infants the effects of H administration on the glycemic response to L-alanine in SGA infants.

MATERIAL AND METHODS

Subjects Thirty-one newborn infants were studied during the first three days of life (Table 1). All study procedures were approved by the ethic committee of the Department of Neonatology and of the Unité de Recherche U34 Hôpital Debrousse. All infants were SGA, their birthweight being under the 10th percentile of the intrauterine growth curve (13). Their gestational age was determined by menstrual history and by clinical assessment according to the method of Dubowitz et al. (13). Infants with Apgar scores lower than five and infants whose mothers had previously received betamethasone injections were excluded. Feeding was started at the age of six hours with 40 to 60 ml/kg/24 h the first day, 60 to 80 ml/kg/24 h the second and 80 to 100 ml/kg/24 h

Table 1 Main clinical data

Small for date infants	No	Gestational age (weeks)	Birth weight (g)	Postnatal age (hours)	Complications
Without hydrocortisone	17	38.3 (35-42)	1 840 (1 590-2 250)	41 (25-82)	Toxemia=4 Twins=7
With hydrocortisone	14	37.8 (35-42)	2 000 (1 640-2 360)	43.8 (34-56)	Toxemia=5 Twins=2 Caesarean section=1

Mean ranges in parentheses

the third day with a commercial milk providing 67 cal/100 ml and lactose 7.25 g/100 ml

In addition all infants were infused at entrance into the unit with 10% glucose in order to achieve with feeding a daily input of 10 g/kg glucose. The infants were separated into two groups: group I 17 control SGA infants and group II 14 SGA infants with hydrocortisone administration. The main clinical data of these groups are shown in Table 1. Five infants developed hypoglycemia (i.e. plasma glucose levels lower than 1.65 mmol/l) in group I and four in group II. At the end of the glucose infusion plasma glucose levels were similar in both groups (mean and ranges): in group I 5.06 mmol/l (1.81-10) and in group II 5.33 mmol/l (2.25-9.07).

Procedures. Feeding and glucose infusion were discontinued three-four hours prior to the test and isotonic saline solution was infused at the rate of 0.5 ml/min. In group II hydrocortisone hemisuccinate (10 mg/kg) was injected intravenously three hours before alanine administration. L-Alanine in a sterile solution prepared in

the laboratory of the hospital was administered intravenously at the dose of 150 mg/kg of body weight into a scalp vein over a period of 30 to 60 sec. Blood samples were collected through an arterial umbilical catheter previously inserted at 10, 30, 30 and 40 min only blood glucose, pyruvate and lactate were studied at 50 min to reduce the number of blood samples.

Measurements. 0.1 ml of blood was collected for glucose determination by a glucose-oxidase method (Beckman glucose Analyser). Specimens of 0.5 ml collected in heparinized microtubes were immediately deproteinized by perchloric acid (0.6 M) and blood pyruvate, β -hydroxybutyrate and lactate were determined immediately by an enzymatic method (25-27). Blood samples were collected in heparinized tubes and in tubes with EDTA and trasylol for insulin and glucagon determinations (1.5 ml). The tubes were kept in ice and immediately centrifuged at 4°C. Plasma was removed and frozen until assayed. The samples were analysed for insulin by the method of Hales & Randle (10) and for glucagon by a radioimmunoassay which did not cross with purified gut glucagon (11). The results were analysed by means of the Fisher test followed by the Student's and paired-*t* test.

RESULTS

Base line levels. Plasma glucose concentration was higher in group II than in the control group I (mean \pm S.E.M.) 4.78 ± 0.2 mmol/l vs 2.88 ± 0.2 mmol/l ($p < 0.01$). Plasma concentrations of glucagon and insulin were lower after H administration 62.25 ± 6.6 vs 81 ± 6.6 μ mol/l in group I ($p < 0.05$) and 42 ± 0.5 vs 12 ± 0.1 μ U/ml in group I ($p < 0.05$) respectively. But the insulin/glucagon molar ratio was not significantly different in group I (1.09 ± 0.13) and group II (0.82 ± 0.08) ($p > 0.05$). The basal level of blood pyruvate was lower after H administration 38.42 ± 7.52 μ mol/l than in group I 89.73 ± 11.81 μ mol/l. No significant differ

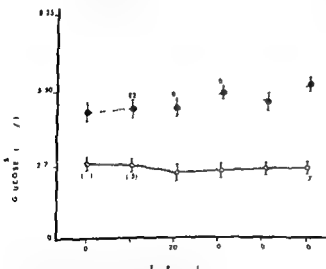


Fig. 1 Effect of L-alanine (150 mg/kg) on plasma glucose concentration (mean \pm S.E.M.) in control SGA infants (O—O) and in group II three hours after hydrocortisone (H) administration (●—●). * — the number of infants is given in parentheses. * significant level over baseline ($p < 0.05$) (paired *t* test).

Table 2 Effect of L alanine (150 mg/kg) on plasma glucagon and insulin concentrations and insulin/glucagon molar ratio in control (group I) and hydrocortisone primed (group II) infants

	Time (min)				
	■	10	20	30	40
Plasma glucagon (pmol/l)					
Group I	■ ± 6.6 (17)	98 ± 10 (14)	113 ± 10 (15)	117 ± 12 (16)	106 ± 13 (16)
Group II	67 ± 6.6 (14)	56 ± 5.7 (11)	69 ± 7.5 (14)	63 ± 5.7 (14)	67 ± 6.0 (14)
Plasma insulin (μU/ml)					
Group I	17 ± 1 (17)	15 ± 7 (14)	14 ± 1 (16)	12 ± 1 (17)	11 ± 1 (17)
Group II	6.4 ± 0.5 (14)	6.9 ± 0.7 (11)	8.0 ± 1 (14)	7.9 ± 1 (11)	7.4 ± 1 (14)
Insulin/glucagon (molar ratio)					
Group I	1.09 ± 0.13 (17)	1.19 ± 0.18 (14)	0.96 ± 0.17 (14)	0.84 ± 0.17 (16)	0.82 ± 0.17 (16)
Group II	0.87 ± 0.08 (14)	0.97 ± 0.08 (11)	0.86 ± 0.08 (14)	0.95 ± 0.09 (11)	0.90 ± 0.08 (14)

Mean ± S.E.M. number in parentheses = number of infants

ence was observed with the B hydroxybutyrate levels $79.2 \pm 12 \mu\text{mol/l}$ in group I and $118 \pm 23 \mu\text{mol/l}$ in group II ($p > 0.05$).

Response to L alanine administration The effect of L alanine infusion on the mean glucose concentration is shown in Fig. 1. A significant rise over the baseline level from 4.78 ± 0.2 to $5.94 \pm 0.2 \text{ mmol/l}$ ($p < 0.01$) was observed after H administration while no significant change (from 2.88 ± 0.2 to $2.59 \pm 0.2 \text{ mmol/l}$) was found in the control group. Intravenous alanine administration produced no significant change in plasma glucagon, plasma insulin concentrations and insulin/glucagon molar ratio (Table 2) or blood lactate and pyruvate concentrations (Table 3) in either group.

DISCUSSION

In a previous study performed exactly under the same conditions (22) we observed that in normal full term infants the intravenous administration of L alanine produced a significant rise of plasma glucose concentrations over the baseline levels in premature infants; it induced a small but significant decrease in plasma glucose concentration which subsequently was found at the baseline level. These effects were observed with an increase in plasma glucagon concentrations but no change of plasma insulin levels in both groups. In the SGA infants of the control group none of these effects were observed since there was no change of plasma glucose, glucagon

Table 3 Effect of L alanine (150 mg/kg) on blood pyruvate and lactate concentrations in control (group I) and hydrocortisone primed (group II) infants

Time (min)	Pyruvate (μmol/l)		Lactate (μmol/l)	
	Group I	Group II	Group I	Group II
0	89 ± 17	38 ± 7	9.7 ± 9.7	3.78 ± 5.94
10	90 ± 13	31 ± 7	3.14 ± 8.40	3.48 ± 4.30
20	91 ± 1	45 ± 7	3.03 ± 9.0	3.57 ± 8.30
30	89 ± 17	3 ± 8	6.0 ± 5.0	7.80 ± 8.30
40	88 ± 14	46 ± 9	7.36 ± 4.40	3.37 ± 8.60
50	87 ± 16	45 ± 10	1.86 ± 3.40	3.47 ± 1.16

Mean ± S.E.M.

Table 1 *Main clinical data*

Small for date infants	No	Gestational age (weeks)	Birth weight (g)	Postnatal age (hours)	Complications
Without hydrocortisone	17	38.3 (35-41)	1 880 (1 590-2 250)	41 (25-82)	Toxemia=4 Twins=2
With hydrocortisone	14	37.8 (35-41)	2 000 (1 640-2 360)	43.8 (34-56)	Toxemia=5 Twins=2 Cesarean section=1

Mean ranges in parentheses

the third day with a commercial milk providing 67 cal/100 ml and lactose 7.25 g/100 ml.

In addition all infants were infused in entrance into the unit with 10% glucose in order to achieve with feeding a daily input of 10 g/kg glucose. The infants were separated into two groups: group I: 17 control SGA infants and group II: 14 SGA infants with hydrocortisone administration. The main clinical data of these groups are shown in Table 1. Five infants developed hypoglycaemia (i.e. plasma glucose levels lower than 1.65 mmol/l) in group I and four in group II. At the end of the glucose infusion plasma glucose levels were similar in both groups (mean and ranges): in group I 5.06 mmol/l (1.81-10) and in group II 5.33 mmol/l (2.25-9.07).

Procedures. Feeding and glucose infusion were discontinued three-four hours prior to the test and isotonic saline solution was infused at the rate of 0.5 ml/min. In group II hydrocortisone hemisuccinate (10 mg/kg) was injected intravenously three hours before alanine administration. L-Alanine in a sterile solution prepared in

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RESULTS

Base line levels. Plasma glucose concentration was higher in group II than in the control group I (mean \pm S.E.M.) 4.78 ± 0.2 mmol/l 2.88 ± 0.2 mmol/l ($p < 0.01$). Plasma concentrations of glucagon and insulin were lower after H administration: 62.25 ± 6.6 vs 81.6 ± 6.6 pmol/l in group I ($p < 0.05$) and 6.42 ± 0.5 vs 12.1μ U/ml in group I ($p < 0.05$) respectively. The insulin/glucagon molar ratio was not significantly different in group I (1.09 ± 0.13) group II (0.82 ± 0.08) ($p > 0.05$). The basal level of blood pyruvate was lower after H administration: $38.42 \pm 7.52 \mu$ mol/l than in group I $89.73 \pm 11.81 \mu$ mol/l. No significant dif-

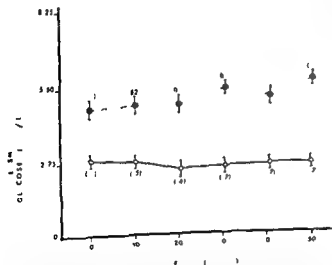


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Langerhans may inhibit insulin secretion such an effect has been demonstrated by Colle & Goldman (1) by in vitro studies on pancreas of human fetuses and premature infants. In addition they showed that H has no effect on glucagon release.

In conclusion this study shows that after administration of pharmacological doses of hydrocortisone an hyperglycemic response to L-alanine is observed without glucagon stimulation in SGA. The occurrence of this hyperglycemic response is probably facilitated by the reduction of peripheral utilization of glucose. These results also suggest that the rise of plasma glucose concentration after alanine administration is due to direct conversion of alanine into glucose. Lower plasma insulin concentrations after H administration may also explain these results.

REFERENCES

- Colle E & Goldman M Effect of glucocorticoids on insulin release from human fetal islets of Langerhans *Diabetes* 25 Suppl 1 359 1976
- Cornblath M & Schwartz M Disorders of carbohydrate metabolism in infancy W B Saunders Philadelphia 1966 p 47
- Cornblath M Wybregt H Baens G S & Klem R I Symptomatic neonatal hypoglycemia: studies of carbohydrate metabolism in the newborn infant VIII *Pediatrics* 33 388 1964
- Dubowitz L M S Dubowitz M & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr* 77 1 1970
- Exton J H Gluconeogenesis *Metabolism* 21 945 1977
- Felg P Polesky T Maruss E & Cahill G F Jr Alanine role in gluconeogenesis *Science* 167 1003 1970
- Fernandez J & Bloom W The intravenous L-alanine tolerance test as a mean for investigating gluconeogenesis *Metabolism* 23 1449 1974
- Fisher H Jr Williams P R Fisher D A Delamater P V Sperling M A & Oh W The effect of oral alanine on blood glucose and glucagon in the human newborn infant *Pediatrics* 56 78 1975
- Gente J C H Warner M Persson H E & Cornblath M Intravenous glucose tolerance plasma insulin free fatty acids and beta hydroxy butyrate in underweight newborn infants *Acta Paed et Scand* 58 481 1969
- Hales C N & Randle P J Immunoassay with insulin antibody precipitate *Biochem J* 83 137 1963
- Haymond A W Karl I E & Paghara A S Increased gluconeogenic substrates in the small for gestational age infant *N Engl J Med* 291 3.2 1974
- Lubchenco L O & Bard H Incidence of hypoglycemia in newborn infants classified by birthweight and gestational age *Pediatrics* 47 831 1971
- Lubchenco L O Hansmann C & Boyd E Intrauterine growth in length and head circumference as estimated from live births at gestational ages from 26 to 47 weeks *Pediatrics* 37 403 1966
- Marco J Calle C Roman D Diaz Fierros M Villanueva M L & Valverde I Hyperglucagonism induced by glucocorticoid treatment in man *N Engl J Med* 288 1 8 1973
- Martin R J Patt J A & Eberhart R J The effects of metyrapone and ACTH on the development of gluconeogenesis in the neonatal pig *Proc Soc Exp Biol Med* 144 603 1973
- Muller W A Faloara U R & Unger R H Jr Effect of alanine on glucagon secretion *J Clin Invest* 50 775 1971
- Munck A Glucocorticoid inhibition of glucose uptake by peripheral tissues: old and new evidence: molecular mechanisms and physiological evidence *Perspect Biol Med* 14 265 1971
- Oh W Baens G S Migeon C J Wybregt S H & Cornblath M Studies of carbohydrates metabolism in the newborn infant V The effect of cortisol on the hyperglycemic response to glucagon *Pediatrics* 30 769 1962
- Paghara A S Karl I E De Vivo M C Feigen R D & Kipnis D M Hypoalaninemia: a concomitant of ketotic hypoglycemia *J Clin Invest* 51 1440 1973
- Price H V Courley T & Cameron E H D Neonatal hydrocortisone administration: effect on cortisol and glucose levels *Biol Neonate* 30 138 1976
- Ruitton A & Frederich F Dosage radioimmunologique spécifique du glucagon pancréatique: Applications chez le nouveau né et l'enfant *Metabolisme et Diabète* 1 143 1975
- Sann L Ruitton A Mathieu M Bourgeois J & Genoud J Effect of intravenous L-alanine administration on plasma glucose, insulin and glucagon, blood pyruvate, lactate and beta hydroxybutyrate concentrations in newborn infants. Study in appropriate and preterm newborn infants *Acta Paediatr Scand* 67 797 1978
- Seitz H J Kaiser M Krone W & Tarnowski W Physiologic significance of glucocorticoids and insulin in the regulation of hepatic gluconeogenesis during starvation in rats *Metabolism* 25 1545 1976
- Shelley H J & Neigan M A Neonatal hypoglycemia *B Med Bull* 23 34 1966
- Tfelt P & Sigaard Anderson O Lactate and pyruvate determinations in 50 µl whole blood *Scand J Lab Invest* 27 15 1971
- Vildnes J & Sovik O Gluconeogenesis in infancy and childhood II Studies of the glucose production from alanine in three cases of persistent neonatal hypoglycemia *Acta Paediatr Scand* 65 307 1976

and insulin concentrations after alanine administration. Our results in these SGA infants agree with the findings of Williams et al (28) with oral administration of alanine. Blood lactate and pyruvate concentrations were measured in SGA control infants to find out whether a partial defect in hepatic gluconeogenesis could be detected (7). However there were no changes of blood lactate and pyruvate concentrations after L-alanine administration. These results do not suggest a defect of gluconeogenesis (7) although a defect in phosphoenolpyruvate carboxykinase cannot be completely ruled out (26).

In the control infants of group I the production of glucose after alanine administration could be balanced by a marked peripheral utilization of glucose; this could explain that plasma glucose concentration did not change after alanine administration. On the contrary, in the SGA infants primed with hydrocortisone (group II) the basal levels of glucose were higher than in group I. These results agree with the hyperglycemic effect of H. As shown by Gentz et al (9) it could be explained by the reduction of peripheral glucose utilization. This could be supported in our study by the depressed plasma insulin levels in group II. However the rise of plasma glucose concentration after alanine administration also indicates that some glucose was produced from alanine.

This study gives also some indications of the process of glucose production in these infants. In adults and children the hyperglycemic response to alanine has been attributed to increased secretion of glucagon (16) and/or to direct conversion of alanine into glucose through gluconeogenesis (6, 19). In the H-primed SGA infants hyperglycemia was induced by L-alanine despite lower levels of plasma glucagon than in the control group and without any change of plasma glucagon concentration or of insulin/glucagon molar ratio. Therefore these results do not support a participation of glucagon to the hyperglycemic response to alanine in H-primed SGA infants.

These data are different from the findings of a study in adults where dexamethasone given for three days induced an increase in plasma glucose and glucagon concentration (14).

Since glycogenolysis was not stimulated through glucagon these data rather suggest that the rise of plasma glucose concentration occurred through gluconeogenesis in these infants. This assumption is supported by the lower concentrations of blood pyruvate in group II (23). This is also supported by the absence of any change in serum insulin and glucagon concentrations after L-alanine administration. Finally, it would be in agreement with studies in neonatal pigs showing that H improves their gluconeogenic capacities (18). A direct effect of H on gluconeogenesis could be limited by the delay of enzyme activation by H. However H stimulates the activity of the gluconeogenic enzymes rather than their synthesis (23). Therefore a direct influence of H on alanine transformation into glucose cannot be excluded in the infants of group II. However this conclusion could be demonstrated in SGA infants only by quantitative measurements which are difficult to realize in newborn infants for technical and ethical reasons. Further studies are required to determine the exact participation of H to these results.

The absence of glucagon and insulin stimulation by alanine in group II is similar to the findings in the control group. The lower levels of plasma insulin and glucagon in group II were surprising results since in adults acute administration of H did not influence the plasma concentrations of insulin and glucagon (14). Hyperglycemia could have precluded the induction of a rise in plasma glucagon as shown by Fiser et al (8). However this effect could not explain the lower level of insulin in group II. Impairment of cellular penetration of glucose by H (6) could account for the absence of insulin response to hyperglycemia but it could not be applied to the lower levels of plasma glucagon. Finally a direct suppressive influence of H on the β cells of

CASE REPORT

A VARIANT OF THE KLIPPEL-TRENAUNAY-WEBER SYNDROME WITH TEMPORAL LOBE ASTROCYTOMA

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ABSTRACT Howitz P, Howitz J and Gjerris F (Departments of Paediatrics, Dermatology and Neurosurgery, University of Copenhagen Rigshospitalet Copenhagen Denmark). A variant of the Klippel-Trenaunay-Weber syndrome with temporal lobe astrocytoma. *Acta Paediatr Scand* 119: 119, 1979. An 8-year-old boy with a variant of the Klippel-Trenaunay-Weber syndrome (KTW syndrome) is described. The hemangiomatous tissue located in the right half of his trunk and extremities was hypoplastic. On the same side, on his face and gingivae the tissue appeared hypertrophic and dental abnormalities were present. Moreover, the patient suffered from psychomotor epilepsy caused by a right-sided temporal astrocytoma. The connection between the KTW syndrome and the neurocutaneous syndromes is discussed.

KEY WORDS Klippel-Trenaunay-Weber, psychomotor epilepsy, temporal lobe astrocytoma, neurocutaneous syndromes.

In 1900 Klippel & Trenaunay described the naevus variqueux osteo hypertrophique syndrome. This description included as main items cutaneous and subcutaneous haemangiomas, phlebectasias and hypertrophy especially of the skeletal bones in the affected areas of the body (5). Some years later Weber reported on a similar entity (10). The close connection between the Klippel-Trenaunay-Weber syndrome (KTW syndrome) and the neurocutaneous syndromes was established early (9).

Subsequently more abnormalities have been added to the KTW syndrome, i.e. macro-syndactyli (6), dental abnormalities (7) and atrophy of the involved areas (4).

The patient described in the following report presents a variant of the KTW syndrome. Apart from dystrophy of the haemangiomatous tissue he suffered from a psychomotor epilepsy caused by a temporal astrocytoma located to the affected side.

CASE REPORT

The patient, an 8-year-old boy, had been afflicted since birth by cutaneous telangiectasia resembling a port wine stain of the right half of his body with the exception of his face. The angiomatous area was hypoplastic, whereas he showed hypertrophy of the right side of his face (Fig. 1). The facial asymmetry was not caused by osseous hypertrophy as ascertained by X ray of the skull. Dental abnormalities and right-sided gingival hyperplasia were present (Fig. 2).

No sign of neurocutaneous disease was apparent. At 5 months of age focal epileptic seizures occurred. Electroencephalography (EEG) showed a right-sided focal pathology, but both the neurological examination and technetium brain scanning were normal. During short-term treatment with diphenylhydantoin the seizures disappeared and the EEG became normal. At 5 years of age psychomotor epilepsy developed and treatment with carbamazepine was initiated.

At the age of eight his epilepsy was aggravating and for the first time he complained of headache. Ophthalmological examination was normal. X ray of the skull showed distension of the sutures but no calcifications. Computer scanning demonstrated a tumour of the right temporal lobe and a carotid arteriography revealed the tumour to be non-vascular. At craniotomy a 5×4×3 cm well-delimited tumour was found in the medial lower part of the right temporal lobe which was removed in toto. Histopathological examination showed an astrocytoma isomor-

- 27 Wildenhoff K E A micromethod for the enzymatic determination of acetate and D-hydroxybutyrate in blood and urine *Scand J Clin Lab Invest* 25 171 1971
- 28 Williams P R Fiser J H Sperling M A & Oh W Effect of oral alanine feeding on blood glucose plasma glucagon and insulin concentrations in small for gestational age infants *N Engl J Med* 292 612 1975
- 29 Wise J K Hendler R & Feig P Influence of glucocorticoids on glucagon secretion and plasma amino acids concentrations in man *J Clin Invest* 57 2774 1973

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Hopital Debrousse
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- ostéo-hypertrophique *Arch Gen Med (Paris)* 3
641 1900
- Lamar L M Farber G A & O Quinn S E
Klippel-Trenaunay-Weber syndrome *Arch Derma-
tol* 91 58 1965
- 7 Miescher G Uber plane Angiome (Naevi hyper-
aemici) *Dermatologica* 106 176 1953
- 8 Peterman A F Hayles A H Dockerty M B &
Love J ■ Encephalotrigeminal angiomatosis
(Sturge-Weber disease) *JAMA* 167 7169 1958
- 9 Teller H & Lindner H Uber Mischformen der
phakomatosen Syndrome von Sturge-Weber und
Klippel-Trenaunay *Z Hautkr* 13 113 1957
- 10 Weber F P Angioma formation in connection with
hypertrophy of limbs and hemihypertrophy *Br J Der-
matol* 19 731 1907

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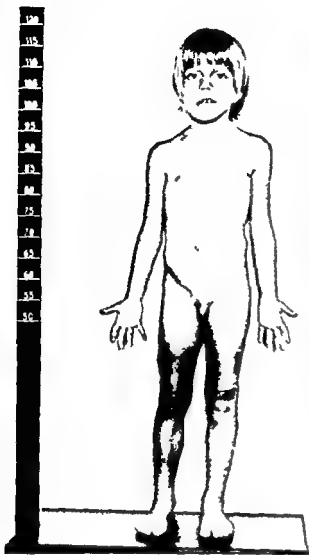


Fig. 1 Right sided hypotrophic port wine naevus in an 8 year old boy

phicum piloides, Kernohan type I. After the operation the psychomotor epilepsy disappeared entirely.

DISCUSSION

A KTW syndrome including dystrophy of the haemangiomatous areas of the body and cases with swelling of one half of the face and dental abnormalities have previously been reported (1, 4, 7). In addition to the above mentioned symptoms, our patient suffered from a psychomotor epilepsy caused by an astrocytoma in the temporal lobe on the same side as the angiomatous tissue.

An atypical manifestation of the Sturge-Weber syndrome might be suggested in spite of the lack of facial port wine naevus (8). How-



Fig. 2 Dental abnormalities and right sided gingival hyperplasia

ever, our patient had no intracerebral calcifications, no ocular involvement and no angioma of the leptomeninges.

Because of the known connection between the KTW syndrome and the neurocutaneous syndromes (phacomatoses) (9) a tumour of the cerebrum might be expected in our patient. The neuropathological pattern of glial proliferation in the phacomatoses results in the possibility of tumours at every level of the nervous system (2, 3). We therefore suggest that the combination of the KTW syndrome and a cerebral astrocytoma, as found in our patient, is an expected variant of the KTW syndrome. To our knowledge, our patient represents a combination of syndromes not previously reported.

REFERENCES

1. Belovic B, Nethercott J & Donsky H J. An unusual variant of Klippel-Trenaunay-Weber syndrome. *Can Med Assoc J* 111: 439, 1974.
2. Canale D, Bebin J & Knighton K S. Neurologic manifestations of von Recklinghausen's disease of the nervous system. *Confin Neurol (Basel)* 24: 359, 1964.
3. Chalhub E G. Neurocutaneous syndromes in childhood. In A L. Prensky (ed.) *Pediatric Neurology: The Pediatric Clinics of North America*. W B Saunders Company, Philadelphia, 1976, p. 509.
4. Ippen H. Quadrantendystrophie mit Gefassanomalien. *Dtsch Med Wochenschr* 98: 687, 1973.
5. Klippel M & Trenaunay E. Du naevus variqueux

- ostéo hypertrophique *Arch Gen Med (Paris)* 3
641 1900
- 6 Lamar L M Farber G A & O'Quinn S E
Klippel-Trenaunay-Weber syndrome *Arch Derma-
tol* 91 58 1965
- 7 Miescher G Über plane Angiome (Naevi hyper-
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(Sturge-Weber disease) *JAMA* 167 7169 1958
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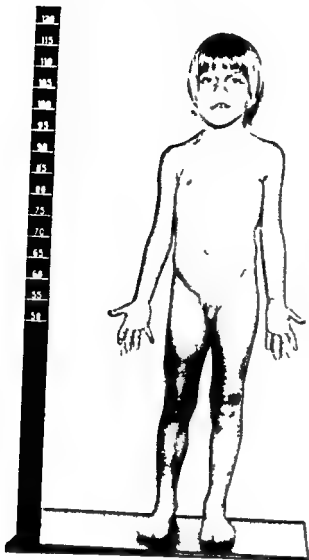


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3. Chalhub E G. Neurocutaneous syndromes in childhood. In: A L. Pinsky (ed.) *Pediatric Neurology: The Pediatric Clinics of North America*. W B Saunders Company, Philadelphia, 1976, p. 509.
4. Ippen H. Quadrantendystrophie mit Gefassanomalien. *Dtsch Med Wochenschr* 98: 687, 1973.
5. Klippel M & Trenaunay P. Du naevus variqueux

CASE REPORT

A VARIANT FORM OF 2 METHYL 3 HYDROXYBUTYRIC
AND 2 METHYLACETOACETIC ACIDURIA

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ABSTRACT Halvorsen S, Stokke O and Jellum E (Institute of Paediatric Research and Institute of Clinical Biochemistry Rikshospitalet University of Oslo Oslo Norway) A variant form of 2-methyl 3-hydroxybutyric and 2-methylacetoacetic aciduria. *Acta Paediatr Scand* 68 123 1979.—A new case of assumed β ketothiolase deficiency excreting 2 methyl 3 hydroxybutyrate and tiglylglycine is described in a 15 year-old boy. The patient presented with episodes of metabolic acidosis following intercurrent infections in the early childhood. After the age of 7 years he has had periods of headache but no acidotic episodes have occurred even during infections. Systematic dietary treatment has not been instituted and the patient is physically and mentally normal. This indicates a mild variant of the β ketothiolase deficiency. Diagnosis of the condition may be obscured by large quantities of ordinary ketone bodies and requires gas chromatographic and mass spectrometric techniques.

KEY WORDS β ketothiolase deficiency 2-methyl 3 hydroxybutyrate tiglylglycine metabolic acidosis ketoacidosis gas chromatography mass spectrometry

The clinical pictures of the 6 previously published patients with 2 methyl 3 hydroxybutyric and 2 methylacetoacetic aciduria (β ketothiolase deficiency) have varied widely. In the patient described by Hillman & Keating (3) vomiting and acidosis started a few days after birth. In the four patients described by Daum et al (1) the symptoms started in the second year of life with episodes of vomiting and metabolic acidosis while in the patient described by Gompertz et al (2) the first and only episode of acidosis occurred at the age of 7 years. Between the ages of 2 and 7 years this patient had presented with frequent episodes of headache, abdominal pain and vomiting but none of these episodes had given any cause for concern.

The patient reported in this paper had episodes of metabolic acidosis between 1 and 4 years of age but later in childhood he developed episodes of headache. The purpose of the present report is to describe the clinical

picture of this variant of the thiolase deficiency and to point out that the heterogeneity in this disease probably is as marked as in maple syrup urine disease (MSUD). Further it is the purpose to describe the pattern of metabolites and some difficulties related to the diagnosis of the disorder. The metabolic steps leading to the formation of the abnormal compounds are shown in Fig. 1.

CASE REPORT

T. K. a boy born February 9 1963 was admitted 4 times to the Department of Pediatrics Rikshospitalet Oslo for coma and/or acidosis. There is no consanguinity between the parents. An older brother is healthy.

His mother, grandmother and other female members of the mother's family have hemicrania. None have had periodic vomiting in childhood or other known metabolic diseases.

Pregnancy, delivery and neonatal period were uneventful. The psychomotor development was within normal limits.

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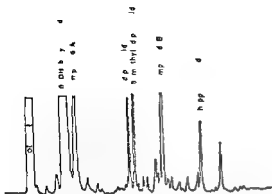


Fig 2 Gas chromatograph separation of organic acids in the urine of the patient. The urine was acidified, extracted with diethyl ether and the organic acids were converted into methyl esters with diazomethane prior to separation on a 6 ft \times 1/4 in glass column filled with 10% OV 17 on Gas Chrom Q. The temperature was programmed from 80 to 780 at a rate of 6 per minute.

ing a urine sample collected during a period of severe metabolic acidosis (second admission). The presence in the urine of large quantities of β -hydroxybutyric acid (Fig 2) was evident. Elevated amounts of adipic acid was also noted (Fig 2) as is usual in ketoacidotic patients (6). In addition to the above metabolites the *propositus* presented with abnormal compounds (denoted A and B in Fig 2) in his urine. The mass spectrum of compound A (Fig 3 top) suggested the structure to be 2-methyl-3-hydroxybutyric acid (methyl ester). In order to verify this structure the authentic compound was synthesized from acetaldehyde and 2-bromopropionic acid using a classical Reformatsky reaction. The synthetic γ -methyl-3-hydroxybutyric acid methyl ester co-chromatographed with compound A from the patient using two different gas chromatography columns (10% OV 17 and 8% BDS). Furthermore the mass spectrum of authentic 2-methyl-3-hydroxybutyric acid methyl ester and of compound A were identical (Fig 3). Thus it was established that the patient excreted considerable quantities of γ -methyl-3-hydroxybutyric acid in his urine.

We also looked carefully for the corresponding keto acid (methylacetoacetic acid) using gas chromatography and mass spectrometry without finding this metabolite. Even when the methoxime-methyl esters were prepared directly from the patient's urine we failed to find the methylacetoacetic acid.

The mass spectrum of compound B suggested its structure to be either tiglylglycine or 3-methylcrotonylglycine. These compounds have rather similar mass spectra as shown in Fig 4. However the relative intensities of some of the fragments differ and it is clearly seen that the unknown compound B and authentic tiglylglycine had identical mass spectra, both being slightly different from that of 3-methylcrotonylglycine methyl ester. Final verification of the identity of compound B was done by isothermal gas chromatography using a polar column (BDS) which

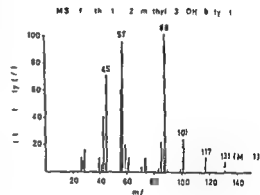
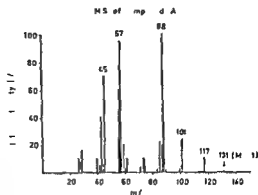


Fig 3 Mass spectra of compound A and of authentic γ -methyl-3-hydroxybutyric acid (methyl esters).

in contrast to the less polar columns (OV 17, SE 30) readily separates tiglylglycine and 3-methylcrotonylglycine methyl esters. As shown in Fig 5 the unknown compound B and tiglylglycine methyl ester co-chromatographed completely, eluting well ahead of the methyl ester of 3-methylcrotonylglycine. Again the mass spectra of compound B and tiglylglycine (methyl ester) were identical, thus establishing the structure of the second abnormal metabolite in the urine of the patient.

Quantitative analyses of tiglylglycine and 2-methyl-3-hydroxybutyric acid were carried out by means of gas chromatography. Glutamic acid, which was not present in native urine, was added as internal standard. Repeated extractions of the acidified urine samples with methyl acetate were carried out before conversion into the methyl esters and separation in the GC. Table 1 shows that approximately equal quantities of tiglylglycine and γ -methyl-3-hydroxybutyric acid were excreted in the urine of the patient. The amounts present in the urines varied from about 0.10 mg per g of creatinine in good clinical phases up to 3 mg per g of creatinine during crisis with metabolic acidosis. Neither the parents nor a healthy brother excreted detectable amounts of the two metabolites which are not present normally.

The concentrations of amino acids in serum and urine have been within normal limits except for an increased

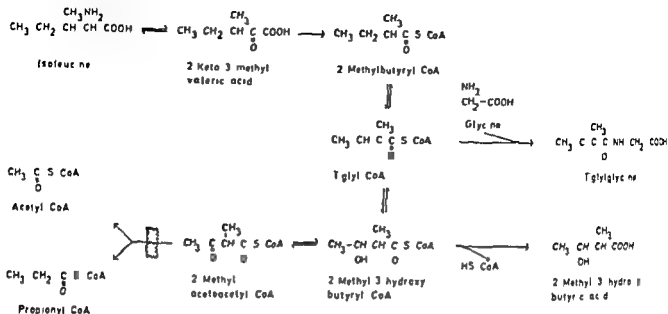


Fig. 1 Metabolic fate of isoleucine. A block is indicated at the level of β -ketothiolase resulting in accumulation of tiglylglycine and 2-methyl 3-hydroxybutyric acid.

First admission Fourteen months old he had as others in the neighbourhood diarrhea and vomiting diagnosed as gastroenteritis. During the first 2 days of his illness he was conscious but on the third day he went into sopor which gradually developed to coma. On admission to Rikshospitalet he was in deep coma and did not react to painful stimuli. The respiration was deep frequent and with a sweet odour from the expired air. He was pale dehydrated and hypotonic. The tendon reflexes were weak. He was restless moved his arms and hands constantly and gave the impression of encephalitis.

The laboratory investigations revealed a marked metabolic acidosis with an arterial blood pH of 6.82 and both standard bicarbonate and P_{CO_2} were very low. The serum electrolytes were normal on admission. He was treated with lactate for 24 hours without success. He was then given THAM and bicarbonate and the acidosis improved and was normalized 72 hours after admission. During the following days the clinical condition slowly improved but it was apparent that the encephalopathy continued for a long period following normalization of the acidosis. EEG was markedly pathological with generalized dysrhythmia.

The urine showed proteinuria and ketonuria. The 2,4-dinitrophenylhydrazine reaction was positive.

During the following year the mother observed deep respiration during periods of upper respiratory infections and fever but on these occasions the patient improved without further treatment.

Second admission At 31/3 years of age he was re-admitted for measles and because he had deep and frequent respiration and sopor. Blood pH was 7.05 standard bicarbonate 8 mmol/l and P_{CO_2} 17 mmHg. This time he was treated with THAM and bicarbonate from the beginning and within 24 hours the acidosis was normalized. The sweet odour from the respiratory air was not noted this time.

Third admission 3 1/2 years old he had an upper respiratory infection and slight changes in respiration. On admission there were no objective signs of acidosis and the pH and standard bicarbonate were only slightly reduced.

Fourth admission At 4 years of age he was admitted the last time for a suspected acidotic episode when he had mumps and a generalized lymph node enlargement which was not well explained. He was conscious and had no respiratory changes. pH was 7.44 standard bicarbonate 18 mmol/l and P_{CO_2} 21 mmHg.

During the following years he had no episodes of acidosis even when suffering from upper respiratory infections with temperatures in the range of 39°C. From the age of 7 years he had episodes with headache. The headache usually came in the morning and lasted 3–4 hours. It was localized in the front in the midline and usually terminated with vomiting. Neurological and ophthalmological examination did not reveal any pathological findings. The patient is now 15 years old and both mentally and physically normal.

Identification of abnormal compounds in the urine of the patient

Urine samples collected during the clinical episodes were examined using gas chromatography (GC) and mass spectrometry (MS) (4). The acidified urine was extracted with diethyl ether and the extract was methylated with diazomethane. An aliquot of the organic acid methyl esters was injected into a Varian CH7 combined GC/MS (Varian MAT Bremen Germany). The GC column (6 ft \times 1/8 in) was filled with 10% OV 17 on Gas Chrom Q or with 8% BDS on Chromosorb W. The GC/MS was connected on line to a computer system (Varian Spectro-system 100 MS).

Fig. 2 shows the gas chromatogram obtained by analysis

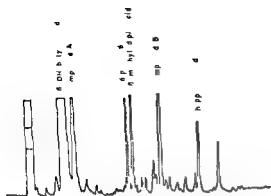


Fig 2 Gas chromatographic separation of organic acids in the urine of the patient. The urine was acidified, extracted with diethyl ether and the organic acids were converted into methyl esters with diazomethane prior to separation on a 6 ft \times 1/4 in glass column filled with 10% OV 17 on Gas Chrom Q. The temperature was programmed from 80° to 80° at a rate of 6° per minute.

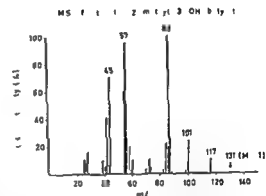
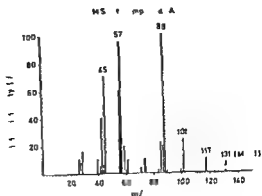


Fig 3 Mass spectra of compound A and of authentic 2-methyl 3-hydroxybutyric acid (methyl esters).

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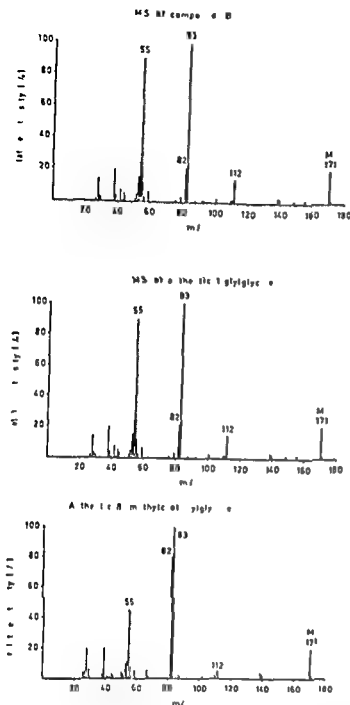


Fig. 4 Mass spectra of compound B of authentic L-glyglycine and of 3-methylcrotonylglycine

glycine level 4.3 $\mu\text{mol}/\text{mg}$ creatinine in the urine sample from the first admission

DISCUSSION

The clinical picture of our patient is characterized by a few episodes of severe metabolic acidosis in infancy with symptoms and signs mimicking those of encephalitis. Apart from

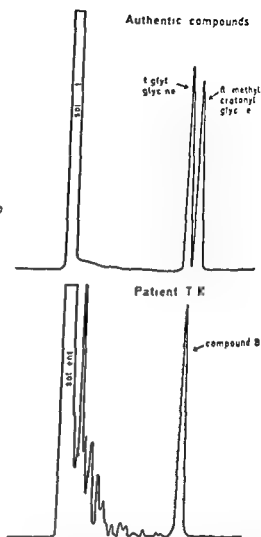


Fig. 5 Isothermal gas chromatographic separation of L-glyglycine and 3-methylcrotonylglycine (top) and of the urinary organic acids in the patient (bottom). The methyl esters were prepared as in Fig. 2. The GC column (6 ft \times 1/4 in) was filled with 8% BDS on Chromosorb W and the column temperature was kept constant at 170°C.

these episodes which were precipitated by gastroenteritis and upper respiratory tract infections, the patient has developed normally. From 7 years of age he has occasionally had periods with headache.

Various 5-carbon fatty acids, e.g. isovaleric and tiglic acids, and several of the corresponding hydroxy acids have been found to exert encephalopathic effects in rabbits (7). Although no data exist for the toxicology of the metabolites found in our patient, it is reasonable to believe that they are at least in part responsible for the metabolic encephalopathy present.

Table 1 Concentration of abnormal metabolites in the urine related to the excretion of creatinine

At 11.5/1 years of age the creatinine excretion was 665 mg/4h

Age (years)		methyl 3 hydroxy butyric acid (mg/g creat)	Tiglyl glycine (mg/g creat)
1 1/2	After treatment	0.51	0.8
3 4/1	Day of admission	2.94	1.01
	2nd day	1.33	1.39
	3rd day	1.34	2.40
	4th day	0.74	0.67
		0.48	0
11 5/12		0.15	0.18
11 7/12		0.20	0.10

ache might possibly be a late complication of this metabolic encephalopathy. It could also be caused by an acute increase in the level of the abnormal metabolites. However, no increased excretion of the metabolites could be found in the urine in relation to the headache making this hypothesis less likely.

The 7 cases with this disease published so far show a wide variation of severity from life threatening acidosis shortly after birth to one single episode of acidosis as late as at 7 years of age. The situation is thus similar to that of MSUD where there are several variants and where each case or family almost has its own clinical picture. This indicates that 2-methyl 3-hydroxybutyric aciduria must be suspected in every case of chronic or intermittent acidosis in infancy or childhood. In the individual patient the episodes may also vary markedly and as in MSUD it appears as if the most severe episodes occur following gastroenteritis.

Although these patients have a sweet odour it is much less characteristic than the curry-like odour of MSUD or that of isovaleric acidemia. By the standard clinical chemical urine analyses it is impossible to distinguish between an ordinary ketoacidosis with the excretion of 3-hydroxybutyrate, acetoacetate and acetone and the present disorder with

the excretion of the corresponding 2-methyl acids. At present a gas chromatographic analysis of either urine or serum is needed in order to make the diagnosis.

Differences in the redox situation in the cells may give rise to different ratios between hydroxy and keto acids. Our patient had apparently displaced the redox state in the direction of the reduced form explaining the absence of both β -methylacetoacetate and acetoacetate.

Even by gas chromatography the diagnosis of a β -ketothiolase deficiency may be difficult. The chromatographic properties of 3-hydroxybutyrate and 2-methyl 3-hydroxybutyrate are closely related making the compounds difficult to separate from each other. Patients with ordinary ketoacidosis excrete small amounts of 2-methyl 3-hydroxybutyrate in addition to the well-known ketone bodies (5). During the acute episodes our patient with a probable β -ketothiolase deficiency excreted larger amounts of 3-hydroxybutyrate than of the typical metabolite 2-methyl 3-hydroxybutyrate. This points to some sort of mutual inhibition of the corresponding enzymic steps in the two pathways. However, the finding of large amounts of 2-methyl 3-hydroxybutyrate together with tiglylglycine is a characteristic feature. In addition, these metabolites are present between the acute episodes also when the ordinary ketone bodies are lacking.

REFERENCES

- Daum H S, Scriver C R, Mamer O A, Delvin E, Lamm F & Goldman H. An inherited disorder of isoleucine catabolism causing accumulation of α -methylacetoacetate and α -methyl β -hydroxybutyrate and intermittent metabolic acidosis. *Pediatr Res* 7: 149, 1973.
- Gompertz D, Saudubray J M, Charpentier C, Bartlett K, Goodey P A & Draffan D H. A defect in L-isoleucine metabolism associated with α -methyl β -hydroxybutyric and α -methylacetoacetic aciduria. Quantitative *in vivo* and *in vitro* studies. *Clin Chim Acta* 57: 69, 1974.
- Hillman R H & Keating J P. Beta-ketothiolase deficiency as a cause of the "ketotic hyperglycemia syndrome". *Pediatrics* 53: 771, 1974.

- 4 Jellum E, Stokke O & Eldjarn I. Combined use of gas chromatography-mass spectrometry and computer in diagnosis and studies of metabolic disorders. *Clin Chem* 18: 800, 1972.
- 5 Landraas S. Accumulation of 3-hydroxyisobutyric acid, 3-methyl-3-hydroxybutyric acid and 3-hydroxyisovaleric acid in ketonacidosis. *Clin Chim Acta* 64: 143, 1975.
- 6 Pettersen J F, Jellum E & Eldjarn I. The occurrence of adipic and suberic acid in urine from ketotic patients. *Clin Chim Acta* 38: 17, 1972.
- 7 Teychenne P I, Walters I, Clavert I F, Calne D B, Price J, MacGillivray B B & Gompertz D. The encephalopathic action of five-carbon atom fatty acids in the rabbit. *Clin Sci Mol Med* 50: 463, 1976.

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CASE REPORT

THE ROLE OF ZINC IN TOTAL PARENTERAL NUTRITION

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ABSTRACT Principi N Giunta A and Gervasoni A (Department of Paediatrics (II) of Milano University Medical School Milano Italy) The role of zinc in total parenteral nutrition *Acta Paediatr Scand* 68 129 1979 —Zinc deficiency was observed in an infant receiving total parenteral nutrition (TPN) for chronic intractable diarrhoea. Clinical findings included low zinc plasma levels skin lesions and loss of all the advantages of TPN such as weight gain serum proteins and albumin increase and normalization of intestinal mucosa. Oral administration of zinc sulphate was the decisive factor making possible both the improvement of clinical and laboratory findings and alimentation by natural route.

KEY WORDS Zinc deficiency total parenteral nutrition

Total parenteral nutrition (TPN) may ensure excellent results only if the infusion contains all the needed nutrients in appropriate amounts (5). A case we recently observed demonstrates the critical role of an adequate zinc supply.

MATERIALS AND METHODS

The patient

A 5-month-old boy was born to healthy unrelated parents after a full term uneventful pregnancy. He weighed 3.4 kg. Neonatal period was normal and he was fed cow's milk formula. Diarrhoea 8 to 15 times per day began at one month of age. Dietary and antibiotic therapy was unsuccessful. At 7 months and 15 days of age he was admitted to our department. Physical examination revealed a poorly nourished infant weighing 3.7 kg and measuring 56 cm. The circumference of his head was 36 cm. Repeated stool cultures revealed no pathogens. During the first 40 days a lactose free diet with soya milk peripheral intravenous infusions of glucose and aminoacids and antibiotics were given without effect on his diarrhoea. At the end of this period the body weight was 3.3 kg.

TPN was then started. About 150 ml/kg of a prepared fat free solution were administered daily through a silastic catheter placed in the superior vena cava. Total caloric intake was about 1.0 calones/kg/die. protein administration 3 g/kg/die and zinc supply 40 µg/kg/die. Essential fatty acids were ensured with daily application of sun flower seed oil to the skin. Plasma or when necessary fresh blood was transfused once weekly at the dosage of

70 ml/kg. A sharp and constant increase of body weight along with the cessation of the diarrhoea was observed. At the end of the first month of TPN the weight increase stopped and skin lesions appeared. They started as a moist eczematoid area in the nasolabial folds and progressed to crusting followed by bullous or pustular lesions all over the face (Fig. 1a) and around the other natural orifices eventually coalescing to form large erosive areas. Some days later a significant alopecia was also noticed. In this period an attempt of oral feeding with a lactose free cow's milk formula produced voluminous diarrhoea and a loss of weight in spite of the intravenous administration of the same volume of nutritive solution. On the 51st day of TPN oral administration of zinc sulphate 25 mg/day was started. Within 5 days a dramatic improvement of skin lesions (Fig. 1b) and a resumption of weight gain occurred. On the 56th day of TPN the dislodgement of the catheter from the cutdown site led to the discontinuation of TPN. A lactose free cow's milk formula was then given while zinc administration at the same dosage continued. Oral feeding was well tolerated, no diarrhoea appeared even though 14 days later zinc supply was discontinued. Skin lesions never recurred and the patient's general condition gradually improved.

Laboratory evaluation

Samples of blood were collected before the beginning of TPN and several times during and after its cessation. In all samples serum total proteins and albumin, zinc and alkaline phosphatase activity were determined. Oral jejunal mucosal biopsies were performed at the beginning and on the 7th day of TPN when the skin lesions appeared and when a good tolerance of oral feeding was obtained. A Crosby-Kugler capsule was used and the specimens stained with hematoxylin and eosin.



Fig 1 (a) Skin lesions after one month of TPN when zinc plasma concentration was $27 \mu\text{g/dl}$ (b) Regression of skin lesions after zinc sulphate oral administration

RESULTS

Before the beginning of TPN serum total proteins and albumin were very low while alkaline phosphatase activity was normal. Zinc plasma level was a little below the normal range (Fig

2). The small bowel biopsy revealed a lamina mucosa with hyperplastic crypts. In the first month of TPN the normalization of serum protein concentration and a significant improvement of the intestinal picture (Fig 3a) was observed. When zinc plasma levels beca

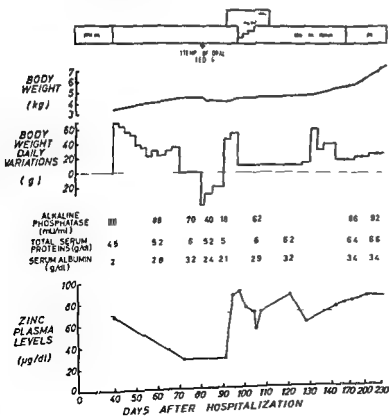


Fig 2 Clinical course and biochemical parameters of the studied patient

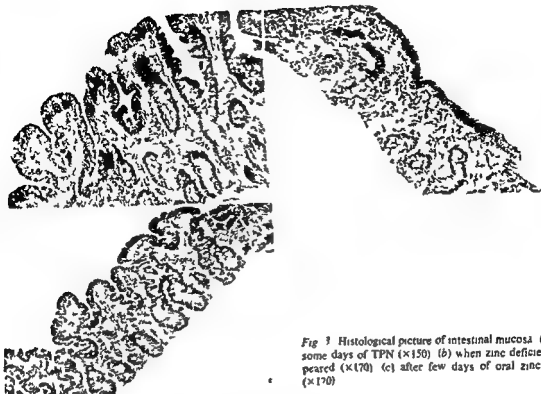


Fig 3 Histological picture of intestinal mucosa (a) after some days of TPN ($\times 150$) (b) when zinc deficiency appeared ($\times 170$) (c) after few days of oral zinc supply ($\times 170$)

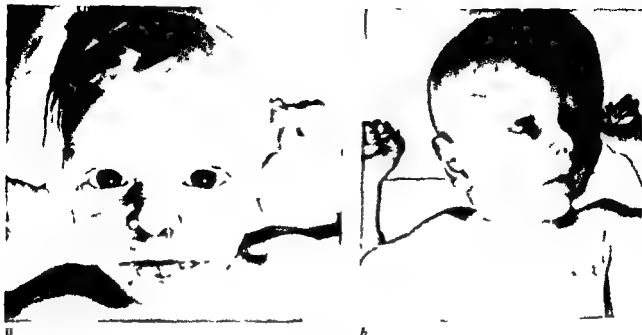
markedly depressed a reduction both of serum protein concentration and of alkaline phosphatase activity was noticed (Fig 2). In addition intestinal mucosal lesions returned similar to those seen when malnutrition was present (Fig 3b). With oral administration of zinc sulphate all the studied biochemical parameters returned to normal values. The last small bowel biopsy demonstrated only a slightly abnormal mucosa (Fig 3c).

DISCUSSION

This case clearly underlines the critical importance of an adequate zinc supply in conditioning TPN efficacy. Clinical and laboratory findings during TPN were as expected for as long as zinc plasma concentration remained near the normal range. When it decreased to a very low level and was accompanied by skin lesions an interruption of weight gain, a drop in serum protein concentration and a reappearance of a flat intestinal mucosa were manifest.

Zinc sulphate administration was the decisive factor making possible both the improvement of clinical and laboratory findings and the alimentation by natural route.

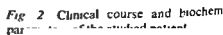
The importance of zinc in nucleic acid metabolism and protein synthesis is well demonstrated in many experimental studies (2, 10, 12) in infants and children an inadequate zinc intake may retard growth, sexual development and wound healing (9, 13) and may induce anorexia, impaired taste perception, pica and lethargy (3, 4). However the best clinical example of zinc deficiency is acrodermatitis enteropathica (8), a disease in which both skin lesions and bowel alterations may be related to a reduced protein synthesis and cellular turnover due to a lack of this element (7). It is reasonable to suggest that the same metabolic impairment may have taken place in this patient leading despite an adequate aminoacid intake to a reduction of serum protein concentration and a regression of intestinal mucosa to atrophy.



4

RESULTS

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CASE REPORT

IMMUNO DEFICIENCY IN SCHWARTZ JAMPEL SYNDROME

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ABSTRACT Mollica F Messina A Stivala F and Pavone L (Departments of Paediatrics and of General Pathology University of Catania Italy) Immunodeficiency in Schwartz Jampel syndrome Acta Paediatr Scand 68 133 1979 —Two sisters born in a consanguineous marriage and affected by Schwartz Jampel syndrome had a complex immunodeficiency involving not only the humoral but also the cellular immune response

KEY WORDS Schwartz Jampel syndrome immunodeficiency

Recently (6) a selective IgA deficiency was found in the serum and external secretions of a girl affected by Schwartz Jampel syndrome (SJS), a rare disease characterized by typical facies, muscle and skeletal abnormalities and growth retardation. Immunological studies performed in two of our patients with this syndrome did not reveal Ig deficiencies but a more complex immunological deficiency.

S P has shown modification of the face by the end of the second year and unsteady gait and progressive loss of muscular strength by the age of 2½ years. She had not experienced frequent infections. At 5½ years she is 103 cm tall (below the 3rd centile) and has a face very similar to

CASE REPORTS

The two patients were sisters born in a consanguineous marriage.

L P has shown progressive modification of her facial appearance and contractures of several muscular groups from the second year of life and progressive muscle weakness and walking difficulty from the third year. No abnormal susceptibility to infections is reported by the parents. On admission the girl is 7 years old. Relevant clinical data are a stature of 110 cm (below the third centile), micrognathia, narrowed palpebral fissures, small mouth, long philtrum (Fig. 1), severe myopia, high pitched voice, pidgeon breast deformity, contractures of several muscular groups, joint limitation, kyphoscoliosis and stuffy nose. A typical myotonic response is found in the electromyogram.

Results of routine laboratory tests are normal except for a low serum albumin/globulin ratio (0.66) and a moderate increase of gammaglobulins (4%). There is lack of response of growth hormone secretion evaluated by radioimmunoassay to arginine and insulin stimulation. An intradermal test with 10 I.U. of PPD (from Berna Institute) is strongly positive although the girl has not been vaccinated against tuberculosis and there is no history of tuberculosis.



Fig. 1 The face of a girl with Schwartz Jampel syndrome

Few data are available on minimum intravenous daily zinc requirement. It is however well known that if during TPN zinc is not added to the nutritive solution a deficiency syndrome may appear (1). The demonstration that in our patient zinc deficiency appeared despite an intake of 40 µg/kg/die suggests that even this amount usually recommended in guidelines for TPN (14) may be insufficient. This conclusion is in complete agreement with the data of Ricour et al (11) and James & MacMahon (6) who demonstrated that during TPN the decline of zinc plasma level can be prevented only by providing term infants and small premature babies with 100 and 200 µg/kg/die respectively.

It is however likely that intravenous zinc requirement may differ significantly from patient to patient according to individual body stores and the degree of anabolism of the first days of TPN. It is important therefore when TPN is performed in infants and children with severe malnutrition to frequently monitor zinc plasma level and eventually increase daily zinc supply if all the ingredients of TPN are to be maintained.

REFERENCES

- 1 Arakawa T, Tamura T, Igarashi Y, Suzuki H & Sandstead H H. Zinc deficiency in two infants during total parenteral alimentation for diarrhea. *Am J Clin Nutr* 29: 197 1976.
- 2 Fosmire G J, Al Ubaidi Y Y & Sandstead H H. Some effects of postnatal zinc deficiency on developing rat brain. *Pediatr Res* 9: 89 1975.
- 3 Hambidge K M, Hambidge C, Jacobs M & Baum J D. Low levels of zinc in hair: anorexia, poor growth and hypogeusia in children. *Pediatr Res* 6: 868 1972.
- 4 Hambidge K M & Silverman A. Pica with rapid improvement after dietary zinc supplementation. *Arch Dis Child* 48: 567 1973.
- 5 Heird W C & Winters R W. Total parenteral nutrition: the state of the art. *J Pediatr* 86: 1975.
- 6 James B E & MacMahon R W. Balance studies of nine elements during complete intravenous feeding of small premature infants. *Aust Paediatr J* 1: 154 1976.
- 7 Kelly H, Davidson G P, Townley R R W & Campbell P E. Reversible intestinal mucosal abnormality in acrodermatitis enteropathica. *Arch Dis Child* 51: 219 1976.
- 8 Moynahan E J. Acrodermatitis enteropathica: a lethal inherited human zinc deficiency disorder. *Lancet* II: 339 1974.
- 9 Pones W H, Henzel J H, Rob C M & Strain W H. Acceleration of healing with zinc sulphate. *Ann Surg* 165: 432 1967.
- 10 Prasad A S & Oberleas D. Thymidine kinase activity and incorporation of thymidine into DNA in zinc-deficient tissues. *J Lab Clin Med* 83: 634 1974.
- 11 Ricour C, Duhamel J F, Gros J, Maziere B & Comar D. Oligo éléments chez l'enfant en nutrition parentérale exclusive. *Arch Franç Ped* 34: 47 1977.
- 12 Sandstead H H, Gillespie D D & Brady R N. Zinc deficiency effect on brain of the suckling rat. *Pediatr Res* 6: 119 1972.
- 13 Sandstead H H, Prasad A S, Schuleri A R, Farid Z, Miale A, Bassilly S & Darby W J. Human zinc deficiency: endocrine manifestations and response to treatment. *Am J Clin Nutr* 70: 477 1967.
- 14 Shils M E. Guidelines for total parenteral nutrition. *JAMA* 270: 1721 1972.

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CASE REPORT

IMMUNO DEFICIENCY IN SCHWARTZ JAMPEL SYNDROME

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ABSTRACT Mollica F, Messina A, Stivala F and Pavone L (Departments of Paediatrics and of General Pathology University of Catania Italy) Immunodeficiency in Schwartz Jampel syndrome. *Acta Paediatr Scand* 68 133 1979.—Two sisters born in a consanguineous marriage and affected by Schwartz Jampel syndrome had a complex immunodeficiency involving not only the humoral but also the cellular immune response

KEY WORDS Schwartz Jampel syndrome immunodeficiency

Recently (6) a selective IgA deficiency was found in the serum and external secretions of a girl affected by Schwartz Jampel syndrome (SJS) a rare disease characterized by typical facies muscle and skeletal abnormalities and growth retardation. Immunological studies performed in two of our patients with this syndrome did not reveal Ig deficiencies but a more complex immunological deficiency.

SJS has shown modification of the face by the end of the second year and unsteady gait and progressive loss of muscular strength by the age of 2½ years. She had not experienced frequent infections. At 5½ years she is 103 cm tall (below the 3rd centile) and has a face very similar to

CASE REPORTS

The two patients were sisters born in a consanguineous marriage.

L.P. has shown progressive modification of her facial appearance and contractures of several muscular groups from the second year of life and progressive muscle weakness and walking difficulty from the third year. No abnormal susceptibility to infections is reported by the parents. On admission the girl is 7 years old. Relevant clinical data are a stature of 110 cm (below the third centile), micrognathia, narrowed palpebral fissures, small mouth, long philtrum (Fig. 1), severe myopia, high pitched voice, pigeon breast deformity, contractures of several muscular groups, joint limitation, kyphoscoliosis and stiff gait. A typical myotonic response is found in the electromyogram.

Results of routine laboratory tests are normal except for a low serum albumin/globulin ratio (0.66) and a moderate increase of gammaglobulins (4.4%). There is lack of response of growth hormone secretion evaluated by radioimmunoassay to arginine and insulin stimulation. An intradermal test with 10 IU of PPD (from Berna Institute) is strongly positive although the girl has not been vaccinated against tuberculosis and there is no history of tuberculosis.



Fig. 1 The face of a girl with Schwartz Jampel syndrome



Fig. 2 The sister of the patient in Fig. 1

that of her sister, with narrowed eyelids, small mouth and sharply pointed chin (Fig. 2). There are thoracic deformity, nasal voice, muscle constrictures, kyphoscoliosis and a hampered gait. Electromyogram shows a typical myotonic response. An intradermal PPD test (10 IU) is negative.

Laboratory findings are normal with the exception of unresponsiveness of serum growth hormone levels to post-arginine and post-insulin hypoglycaemia. The results of the immunological study of the sisters are reported in Table 1. The same tests performed in both parents gave normal results.

DISCUSSION

Selective IgA deficiency is the most frequent immunodeficiency. It has been found in 0.7% of an unselected population (3). It may be found in healthy subjects and in association with various and apparently unrelated disorders. Dr Kirschner & Pachman (6) reported a 3½ year old girl with SJS who showed frequent infections and a selective IgA deficiency. Another subject with this syndrome (1)

Table 1 Immunological studies in 2 sisters with the Schwartz-Jampel syndrome

	Case 1 P.L.	Case 2 P.S.	Normal range in this labor- atory
IgG g/l*	15.01	22.60	8.0-16.8
IgA g/l	1.87	3.33	1.4-4.7
IgM g/l*	3.76	2.88	0.5-1.9
IgE mg/l*	0.35	0.33	0.1-0.6
C3 g/l	0.65	0.60	0.55-1.44
Leukocytes 10 ⁹ /l	4.1	3.3	4.0-9.0
Lymphocytes 10 ⁹ /l	1.64	1.06	1.5-7.7
Concanavalin A stimulation by PHA*	61	57	70-80
Concanavalin A rosette forming cells (T lymphocytes)	16	14.8	55-65
Concanavalin A rosette forming cells (B lymphocytes)	5.5	10.0	15-70

* Immunodiffusion technique using plates from Behringwerke AG (Marburg, Lahn).

* Radioimmunoassay.

* Mononuclear cell cultures were obtained according to Böyum (2). A previously established optimal dose of 0.1 µg/ml of PHA was used for stimulation. E and EA rosette forming cells were evaluated by the techniques of Jondal et al. (5) and of Hallberg et al. (4) respectively.

showed frequent respiratory tract infection but in this patient no immunological studies have been performed. Also in the other reported cases of this syndrome no immunological tests were carried out.

In our patient, while the serum Ig levels were within the normal limits, the number and—to a larger extent—of T lymphocytes evaluated by the E and EA rosette forming cells respectively was definitely below the normal range (see Table 1). Our results suggest that a complex impairment of the immunologic function involving not only the humoral but also the cellular immune response can be found in the Schwartz-Jampel syndrome.

REFERENCES

1. Beighton P. The Schwartz syndrome in South Africa. *Clin Genet* 4: 548, 1973.

Boyum A Separation of lymphocytes from blood and bone marrow *Scand J Clin Lab Invest* 21 Suppl 97 1 1968

Burgo R Severi F Ugazio A & Vaccaro R In *Tema di Deficienza e della immunità specifica Proc 38th Congr Ital Soc Pediatr* Minerva Medica Torino 1976 p 51

Hallberg T Gurner W & Coombs R R A Opsonic adherence of sensitized ox red cells to human lymphocytes measured by rosette formation *Int Arch Allergy* 44 550 1973

Jondal M Holm G & Wigzell H Markers of human T and B lymphocytes 1 A large population of

lymphocytes forming non immune rosettes with sheep red blood cells *J Exp Med* 136 207 1972

6 Kurschner H & Pachman L M IgA deficiency and recurrent pneumonia in the Schwartz-Jampel syndrome *J Pediatr* 88 1060 1976

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REVIEW ARTICLE

INTERACTIONS OF NUTRITION INFECTION AND IMMUNE RESPONSE

Immunocompetence in Nutritional Deficiency Methodological Considerations and Intervention Strategies

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ABSTRACT Chandra R. K. (Department of Pediatrics Memorial University of Newfoundland St John's Newfoundland Canada) Interactions of nutrition infection and immune response Immunocompetence in nutritional deficiency methodological considerations and intervention strategies Acta Paediatr Scand 68 137 1979.—Clinical and epidemiologic data point to a causal interrelationship between nutritional deficiency and infectious illness Both are major contributors to childhood morbidity and mortality particularly in under privileged population groups Energy protein undernutrition and deficiencies of iron folates and pyridoxine depress a variety of immunity functions Delayed hypersensitivity and number of T lymphocytes are consistently reduced In small for-gestation low birth weight infants cell mediated immunity may remain depressed for several years B lymphocytes immunoglobulin levels and antibody responses are generally normal but secretory IgA antibody is reduced Serum complement components are low and there is evidence of in vivo consumption of complement C3 Neutrophil phagocytosis of bacteria and fungi is intact but the next step of intracellular killing is impaired There are changes also in the production of lysozyme and interferon Infection per se results in nutrient losses either actual or by sequestration and produces immunosuppression The correction of postnatal nutritional deficits and/or infection is associated with reversal of immunological functions to normal The interplay of nutrition immunity and infection and its biological implications are described

KEY WORDS Malnutrition immunity lymphocyte function neutrophils complement system infection

The stark spectrum of over 500 million human beings suffering from the multiple erosive effects of undernutrition is a rude eye opener for any serious student of global health problems Malnutrition affects each and every function of the body The most prominent association of nutritional deficiency is with infection The impression is widely held by clinicians that undernutrition increases the frequency and severity of infectious illness At the same time a sick child frequently has no desire to eat may have impaired absorption and has greater metabolic needs thereby worsening his nutritional status Thus infection exacerbates malnutrition and vice versa In this paper recent

data on the mechanisms of interactions between nutrition immunity and infection is reviewed

EPIDEMIOLOGY

Many field surveys have documented a higher frequency of infections particularly gastroenteritis and respiratory disease in the undernourished Prophylactic or therapeutic supplementation of diet is associated with a significant reduction in morbidity These observations have been summarized by Scrimshaw et al (35) Among a group of construction and plantation workers in Indonesia acute and

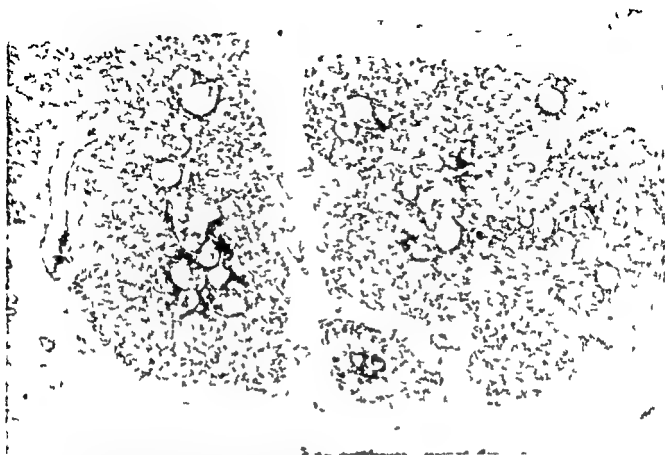


Fig. 1 Thymus in severe energy protein undernutrition. Note depletion of lymphocytes and crowding of Hassall's corpuscles, some of which are degenerating.

chronic infections were more prevalent in iron deficient anemic individuals than in non-anemic controls (1). The effect of iron supplementation on infection related morbidity is variable (12). In moderately severe malnutrition infection is a common primary or contributory cause of death. The Pan American Health Organization study of mortality patterns in Central and South America revealed the presence of undernutrition in 57% of the children who died under 5 years of age, many of them of terminal infection (34). There is a higher frequency of iron deficiency in patients with chronic mucocutaneous candidiasis (29) and in those with recurrent herpes labialis (23). Human data is strongly supported by the almost invariable heightened susceptibility to infectious challenge in laboratory animals (19, 35).

It is essential to point out some of the pitfalls and limitations of the available epidemiologic information on the incidence of infection in malnutrition and vice versa. Most of the studies have conducted one point analysis and there was no longitudinal follow up. Moreover the common occurrence of infection and nutritional deficiency does not necessarily imply a causal relation, but both may have been the result of the same environmental factors such as poverty.

MORPHOLOGICAL CHANGES

In moderately severe malnutrition involution of the thymus is a consistent finding (19, 40). The lymphocytes are fewer in number and Hassall's corpuscles may be degenerated (Fig. 1). In lymph nodes and the spleen the

Table 1 Immunoglobulin containing plasma cells in the jejunum

Values are expressed as number per $6 \mu\text{m} \times 500 \mu\text{m}$ tissue. Mean \pm S.D. is given

Group	IgA	IgM	IgG
Malnourished	67 ± 17	27 ± 9	5 ± 2
Healthy	93 ± 13	17 ± 5	3 ± 1

thymus dependent areas paracortical and perarteriolar regions respectively are depleted of small lymphocytes (19). In a few patients with advanced malnutrition germinal follicles are also small and few. There is little information on the gut associated lymphoid tissues. Tonsils are shrunken (3, 40) and there are fewer IgA producing cells in the jejunal submucosa (Table 1) (14). These alterations in organ size and structure are observed both in acquired postnatal malnutrition (19, 40) as well as in intrauterine growth retardation (17, 33). Comparable changes can be induced in experimental animals deprived of total energy intake proteins vitamin B₆ or iron.

The pathogenesis of such changes in the organs of the immune system is complex. Restricted proliferation of cells, reduced protein synthesis, changes in many hormones, endotoxemia and chaperones may all be responsible. Elevated concentration of free cortisol has been documented in malnourished humans and in deprived experimental animals. It is certain that the additional stress of associated infection contributes to structural alterations in the lymphoid tissues in undernutrition.

IMMUNOCOMPETENCE

Cell mediated immunity and T lymphocytes

Failure to mount delayed cutaneous hypersensitivity reactions in malnutrition has been known for many decades (28). A variety of skin test antigens have been employed including tuberculin, purified protein derivative, *Candida trichophyton*, mumps strepto-

kinase streptodornase, dinitrochlorobenzene and dinitrofluorobenzene (3, 33, 40). In some studies recovery from nutritional deficiency was associated with the ability to show positive skin responses. Impaired delayed hypersensitivity can be the result of thymic dysfunction, decreased macrophage and lymphocyte migration to the local site and reduction in nonspecific cutaneous reactivity either singly or in combination. It is probable that the first two factors are the more important ones in malnutrition.

Depression of cellular immunity in nutritional deficiency has been suggested also by abnormalities in several *in vitro* correlates. For example, rosette forming T lymphocytes in the peripheral blood are almost invariably reduced in proportion (Fig. 2) (5, 13, 25) and absolute number and promptly recover after short term nutritional therapy, often before any obvious clinical or biochemical changes (19). In addition to an actual reduction in the number of T lymphocytes, it has been suggested that cytophilic inhibitors of rosetting with sheep erythrocytes may be at work (15). Among others, IgE (16) and alpha fetoprotein (20) have been reported to be elevated in some malnourished infants and both can inhibit the rosetting process. The unique ability of T cells to respond mitotically to the plant lectin

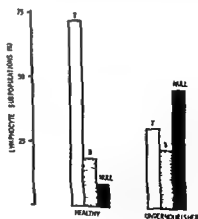


Fig. 2 Lymphocyte subpopulations in the peripheral blood of children with energy protein undernutrition and in controls.

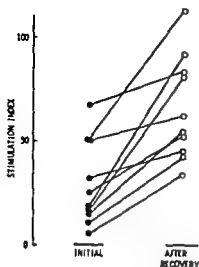


Fig. 3 Lymphocyte stimulation response in 10 children with energy protein undernutrition before and after nutritional recovery. Lymphocytes isolated from the peripheral blood were cultured in the presence of phytohemagglutinin. Stimulation index represents the ratio of counts per minute in PHA containing culture/counts in buffer containing culture.

phytohemagglutinin has been assessed in malnourished groups and found to be reduced (3, 5, 33, 40). This reduction is somewhat greater than might be explained on the basis of fewer T lymphocytes.

The changes in mitogen induced lymphocyte responses in malnutrition are completely reversed to normal on dietary supplementation (5) (Fig. 3). It is important to note that lymphocyte proliferation is reduced even in marginal undernutrition though not consistently (Fig. 4). Besides cellular factors plasma inhibitors of lymphocyte DNA synthesis have been reported in kwashiorkor and marasmus (15, 31). In many such studies the influence of associated infection has been impossible to rule out.

Alterations in lymphocyte subpopulations and in the *in vivo* and *in vitro* delayed hypersensitivity responses have been noted not only in acquired postnatal energy protein malnutrition but also in small for gestation low birth weight infants (11, 29) and in deficiencies of isolated nutrients such as iron (7), folates (27) and vitamin B₆.

In nutritional deficiency there is a proportionate increase in the number of null cells

which do not bear the conventional surface markers of T or B lymphocytes (Fig. 2). The functional attributes of null cells are not entirely clear but preliminary information suggests that they can exert cytotoxic and suppressor effects (13). Further studies on defining the function of these cells are required.

B lymphocytes, immunoglobulins and antibodies

Immunoglobulins and specific antibodies are produced by plasma cells which represent the maturational end stage of B lymphocytes. In malnutrition the proportion and number of circulating B cells is generally normal (Fig. 2) rarely it may be decreased or increased. Serum levels of all the major classes of immunoglobulins are often high (Table 2); this is considered to be the result of repeated infections experienced by undernourished individuals. We have postulated that recurrent protracted infections of the gastrointestinal and respiratory tract occur partly as a result of impaired mucosal immunity (8) in nutritional deficiency and that such infections as well as constant immunological stimulation by the absorbed macromolecules (food antigens, products of gut bacterial flora, etc.) (9) contribute to the polyclonal hyperglobulinemia. Rarely a young infant with severe energy

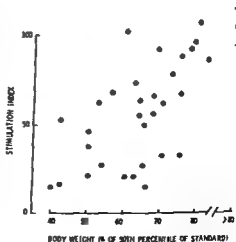


Fig. 4 Lymphocyte stimulation related to deficit in body weight. The mean \pm 1 S.D. of values obtained in nutritionally healthy individuals is shown as a bar.

Table 2. Serum immunoglobulin levels

Group	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	IgD (mg/100 ml)	IgE (U/ml)
Energy protein undernutrition					
With gross infection	2 360±654	268±56	188±43	16.5±7.1	360±110
With demonstrable parasites*	1 435±771	309±79	744±57	79.3±8.9	2 865±581
Without obvious infection or infestation	675±707 ^a	87±73	81±25	3.1±1.9	427±179
Well nourished					
With gross infection	2 895±56	270±38	159±33	11.1±5.3	56±37
With demonstrable parasites	1 435±16	180±53	136±47	7.6±7.9	2 410±736
Without obvious infection or infestation	1 080±19 ^a	110±29	88±21	1.9±0.9	36±21

* Values are expressed as mean ± S.D.

^a Ascans and/or hookworm

Plasma half life of IgG 9.6 days

^a Plasma half life of IgG 31.3 days

Plasma half life of IgG 11.1 days

^a Plasma half life of IgG 19.5 days

From Chandra & Newberne (19)

protein deficiency may have low levels of IgG (3) and other immunoglobulins. This is particularly observed in low birth weight infants who experience a profound and prolonged hypogammaglobulinemia (11). There is paucity of information on the metabolic turnover rates of immunoglobulins in malnutrition. The presence of associated infection accelerates catabolism whereas noninfected children with low serum IgG have a prolonged plasma half life of IgG (Table 2).

Isohemagglutinin titres are normal. Elsewhere we have summarized the published data on the effect of nutritional status on antibody response to natural infection or to administered microbial and chemical antigens (16-19, 21). The difficulties of interpreting most of this information are recognized because of the extreme variations in study protocols. In particular failure to administer a potent antigen or to exclude concomitant infection need emphasis. In general antibody response to most antigens is adequate in the majority of undernourished individuals. Contrarily some particulate antigens e.g. heterologous red blood cells (10) and *Salmonella typhi* (3) may elicit a reduced response.

In kwashiorkor and marasmus secretory IgA concentrations in nasopharyngeal secretions, tears and saliva are low (8, 39, 42) and out of proportion to the slight reduction in

protein levels in general. It is possible that this is the result of reduced synthesis of IgA by the fewer IgA plasma cells in the submucosae (Table 1) or decreased synthesis of secretory component by the atrophied epithelia or both. We have observed reduced secretory IgA antibody response to viral vaccines in a group of children suffering from energy protein malnutrition (8). More studies are required in this important area of host defense.

Antigen nonspecific protective factors

Complement components are vital elements of amplifying the immune response by promoting chemotaxis, immunocoagulation, adherence, opsonization and microbial lysis. Malnutrition causes a reduction in the serum levels of almost all complement proteins (16, 38). The changes are more profound in patients with obvious infection and are reversible on dietary supplementation. Reduced synthesis as well as increased breakdown have been suggested as the underlying mechanisms. Increased consumption is evidenced by the presence of complement C3 degradation products and high titres of immunocoagulation (6).

Opsonic function of plasma is comparable in healthy and undernourished subjects if tested at more than 10% concentration. At lower concentrations of plasma however there is

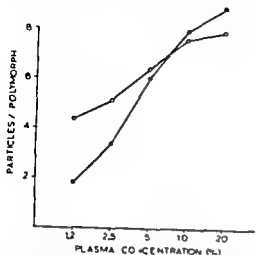


Fig. 5 Opsonic function of plasma. Bacteria and polymorphonuclear leukocytes were incubated in the presence of plasma of malnourished or well nourished children. The number of phagocytosed particles was counted in 100 cells.

a significant difference between the two groups (Fig. 5). This may be relevant and limit the rate of phagocytosis in tissue spaces where complement components and their opsonic factors are present in concentrations much lower than in serum.

The function of polymorphonuclear leukocytes has been evaluated in several groups of undernourished children. The ability to ingest particles is intact but the killing of phagocytosed bacteria is impaired (Fig. 6) (36-38). In an occasional patient virtually no reduction in the count of viable intracellular bacteria is observed paralleling findings in inherited granulocytopenias. However, the microbicidal defect in malnutrition is reversed completely when the individual's nutritional status is restored to normal (19-37). Similar alterations of phagocyte function are documented in iron deficiency (4, 7, 30) and in fetal growth retardation (11). An important methodologic consideration needs mention. The ratio of the number of bacteria to the number of phagocytes is critical. At higher ratios the bactericidal defect of neutrophils from malnourished subjects becomes prominent although it is distinctly demonstrable even at lower ratios of bacteria to phagocytes (Fig. 6).

Chemotactic migration of polymorphs is

fully normal except in individuals with infection (22).

Many other nonspecific protective factors including interferon and lysozyme are altered in nutritional deficiency. These have been reviewed elsewhere (19-35).

EFFECTS OF INFECTION

Infection itself can produce significant changes in nutritional status and immune function. The extent of such alterations is determined by the severity and duration of illness, nature of the microbe and the health status of the individual prior to infection. Infectious disease, particularly febrile illness, increases tissue catabolism and urinary losses; (2) reduces appetite and food intake; it may decrease absorption from the gut and ultimately result in protein losing gastroenteropathy. The combined result of all these processes is further deterioration of nutritional status. It is well known that measles increases losses of nutrients in the feces and the urine, lowers serum albumin concentration (Fig. 7) and may precipitate edema in a child with marginal undernutrition. Infection *per se* depresses

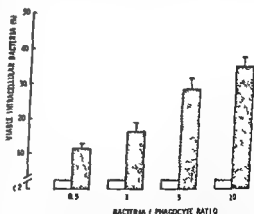


Fig. 6 Bactericidal capacity of polymorphonuclear leukocytes in energy protein undernutrition related to the ratio of the number of bacteria to phagocytes. *Staphylococcus aureus* was the organism used and viable intracellular bacteria were counted at 20 and 140 min of culture and expressed as a percentage ratio. Open columns represent healthy controls and the shaded columns undernourished children. Means and S.E.M. of 15 subjects in each group are shown.

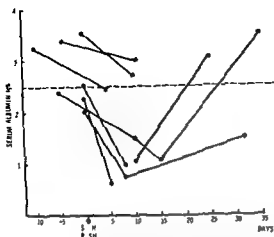


Fig 7 Effect of measles on nutritional status measured by serum albumin concentration. In all patients a significant drop in albumin level occurred. In those with low normal level before the infection there was a more pronounced fall in serum albumin concentration often associated with the appearance of dependent edema. Recovery occurred several weeks following medical nutritional management. From Chandra & Newberne (19).

many aspects of immunity function possibly by the invasion of lymphoid tissues altering the proportion of lymphocyte subpopulations especially suppressor cells, changing hormonal levels, endotoxaemia and by the elaboration of acute phase reactant alpha globulins and chaperones. Measles in the pre exanthematous phase and for several weeks after the appearance of the rash impairs mitogen induced lymphocyte stimulation even though the numbers of T lymphocytes remain unchanged (19). These changes in cellular immunity have prognostic significance (24). Lymphocyte changes also have been described in acute malaria (26). Similarly infection related granulocyte dysfunction may adversely influence the outcome of the infectious process (41).

CONCLUDING REMARKS

The inextricably intertwined problems of undernutrition and infection defy easy analysis in terms of the underlying mechanisms of interactions. Recent studies have revealed im-

paired function of a variety of host protective factors. It is possible that the clinical problem of repeated and severe infections in malnourished persons is the result of a summation of variable individual abnormalities in several key cellular and humoral immunological functions. These changes in immunocompetence may play a critical role in the effectiveness of immunization in the susceptibility to septicaemic spread of infection and in the production of immunopathological disease. Infection *per se* can result in significant deficits in immunity function and in nutrition. At the same time it must be recognized that nutritional status is intricately tied to other health factors and that the roots of malnutrition are largely economic. To tackle this major world affliction we must adopt a portfolio approach of intervention at several levels including socioeconomic development, nutrition education, promotion of breast feeding, agricultural output, immunizations, safe water supply and environmental sanitation (18). The task looks formidable but that is no reason for not trying.

REFERENCES

1. Ba ta M S & Churchill A. Iron deficiency anemia and the productivity of adult males in Indonesia. Staff Working Paper 175. World Bank, Washington D.C. 1974.
2. Bessel W R. Metabolic response to infection. *Ann Rev Med* 26: 9 1975.
3. Chandra R K. Immunocompetence in undernutrition. *J Pediatr* 81: 1184 1972.
4. Chandra R K. Reduced bactericidal capacity of polymorphs in iron deficiency. *Arch Dis Child* 48: 863 1973.
5. Chandra R K. Rosette forming T lymphocytes and cell mediated immunity in malnutrition. *Br Med J* 3: 608 1974.
6. Chandra R K. Serum complement and immunoglobulin in malnutrition. *Arch Dis Child* 50: 25 1975.
7. Chandra R K. Impaired immunocompetence associated with iron deficiency. *J Pediatr* 86: 899 1975.
8. Chandra R K. Reduced serum and secretory antibody response to live attenuated measles and poliovirus vaccines in malnourished children. *Br Med J* 11: 583 1975.
9. Chandra R K. Food antibodies in malnutrition. *Arch Dis Child* 50: 537 1975.

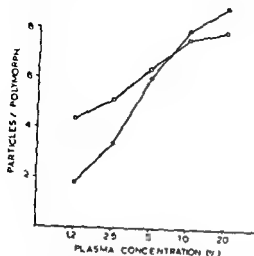


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Infection itself can produce significant changes in nutritional status and immunity function. The extent of such alterations is determined by the severity and duration of illness, nature of the microbe and the health status of the individual prior to infection. In infectious disease, particularly febrile illness, increases tissue catabolism and urinary losses (2), reduces appetite and food intake and may decrease absorption from the gut or actually result in protein losing gastroenteropathy. The combined result of all these processes is a further deterioration of nutritional status. It is well known that measles increases losses of nutrients in the feces and the urine, lowers serum albumin concentration (Fig. 7) and may precipitate edema in a child with marginal undernutrition. Infection *per se* depresses

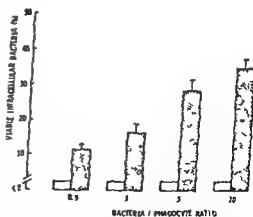


Fig. 6 Bactericidal capacity of polymorphonuclear leukocytes in energy protein undernutrition related to the ratio of the number of bacteria to phagocytes. *Staphylococcus aureus* was the organism used and viable intracellular bacteria were counted at 20 and 140 min of culture and expressed as a percentage ratio. Open columns represent healthy controls and the shaded columns undernourished children. Means and S.F.M. of 15 subjects in each group are shown.

NEW BOOKS RECEIVED

- S S Gellis & B M Kagan *Current Pediatric Therapy* vol 8 879 pp W B Saunders Philadelphia London Toronto 1978 £ 3 00 ISBN 0-716-4089-3
- J H Hanshaw & J A Dudgeon *Viral Diseases of the Fetus and Newborn* (Vol XVII in the series Major Problems in Clinical Pediatrics) 347 pp illus W B Saunders Co Philadelphia London Toronto 1978 £14 00 ISBN 0 7 16-4500-3
- A Moragas A Ballabriga & M T Vidal *Atlas of Neonatal Histopathology* 130 pp illus W B Saunders Co Philadelphia London Toronto 1977 £43 75 ISBN 0-716-6541-X
- Jean de Grouchy & Catherine Turleau *Clinical Atlas of Human Chromosomes* 319 pp illus John Wiley & Sons New York Chichester Brisbane Toronto 1977 £ 4 70 ISBN 0-471-01704 3
- Bernbeck & Sinios *Neuro-Orthopedic Screening in Infancy* Schedules Examinations and Findings 1 0 pp illus Urban & Schwarzenberg Munchen 1978 DM 8 - £7 70 \$13 50 ISBN 3 541 70 31 1
- Derrick B Jelliffe & E F Patrice Jelliffe *Human Milk in the Modern World* 500 pp illus Oxford University Press 1978 £15 00 ISBN 0-19 64919 1
- David J Boulton (ed) *Serotonin in Mental Abnormalities* 316 pp John Wiley & Sons Chichester New York Brisbane Toronto 1978 £13 50 ISBN 0-471 99401-0
- L Gedda & H Parisi (eds) *Auxology Human Growth in Health and Disorder* (Serenio Symposium no 13) 319 pp illus Academic Press Inc London 1978 £11 0 5" 00 ISBN 0-17 79050-2
- G Chumelleo & Z Laron (eds) *Recent Progress in Pediatric Endocrinology* Proceedings of the Sereno Symposia Vol 17 325 pp Academic Press London New York San Francisco 1977 £10 00 \$19 50 ISBN 0-1 173 50-9
- Bernbeck & A Sinios *Neuro-Orthopedic Screening in Infancy* Schedules Examinations and Findings 1 0 pp illus Urban & Schwarzenberg Baltimore Munchen 1978 US\$13 50 ISBN 3 541 7031 1
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- Noeman A Beischer & Eric V Mackay *Obstetrics and the Newborn* 53 pp illus Holt Saunders Pty Ltd 1976 £8 50 ISBN 003 900051-6
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- H J Jelliffe & J P Stanfield (eds) *Diseases of Children in the Subtropics and Tropics* 7nd ed 1049 pp illus Edward Arnold (Publishers) Ltd 1978 £25 00 ISBN 0-7131-4777-4
- The Fetus and Birth* Ciba Foundation Symposium 47 481 pp Elsevier Excerpta Medica/North Holland 1977 No price given ISBN 0-444 15755 5
- Schröder & Brandt *Longitudinalstudie über Wachstum und Entwicklung von früh und reifgeborenen Kindern von der Geburt bis zum 6 Lebensjahr* 56 Seiten illus DM 13 - Westdeutscher Verlag GmbH 1977 ISBN 3 531-07691 7
- J Borms & M Hebbelink (eds) *Pediatric Work Physiology* Vol II In Medicine and Sport Series (ed E Jökl & H Hebbelink) 178 pp illus S Karger Basel Munchen Paris London New York Sydney 1978 US\$39 00 ISBN 3 8055 7866-3
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- A K K Kreutner & D R Hollingsworth *Adolescent Obstetrics and Gynecology* 658 pp Year Book Medical Publishers Inc Chicago London 1978 \$50 50 ISBN 0-8151 5500-0
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- J L Melnick (ed) *Progress in Medical Virology* Vol 4 4 pp illus S Karger Basel Munchen Paris London New York Sydney 1978 US\$54 00 ISBN 3 8055 810-8
- H Homewood Nixon (ed) *Operative Surgery* 3rd edition 489 pp illus Butterworths London Boston 1978 £47 00 ISBN 0-407-00635-6

- 10 Chandra R K Antibody formation in first and second generation offspring of nutritionally deprived rats *Science* 190 289 1975
- 11 Chandra R K Fetal malnutrition and postnatal immunocompetence *Am J Dis Child* 129 450 1975
- 12 Chandra R K Iron and immunocompetence *Nutr Rev* 34 129 1976
- 13 Chandra R K Lymphocyte subpopulations in malnutrition Cytotoxic and suppressor cells *Pediatrics* 59 423 1977
- 14 Chandra R K Nutrition dietary factors and immunology of the gut In *Tripartite Symposium on Gastroenterology* City University London 1977
- 15 Chandra R K Cell mediated immunity in fetally and postnatally malnourished children from India and Newfoundland In R M Suskind (ed) *Malnutrition and the immune response* Raven Press New York 1977 p 111
- 16 Chandra R K Immunoglobulins and antibody response in malnutrition In R M Suskind (ed) *Malnutrition and the immune response* Raven Press New York 1977 p 155
- 17 Chandra R K Ontogenetic development of the immune system and effects of fetal growth retardation *J Perinat Med* 7 1979 In press
- 18 Chandra R K Nutritional deficiency and susceptibility to infection Epidemiological clinical data immune responses and effects of breast feeding transfer factor and levamisole *Bull WHO* 56 1978 In press
- 19 Chandra R K & Newberne P M *Nutrition immunity and infection Mechanisms of interactions* Plenum New York 1977
- 20 Chandra R K & Bhujwala H A Elevated a fetal protein and impaired immune response in malnutrition *Int Arch Allergy Appl Immunol* 53 80 1977
- 21 Chandra R K Chakraborty S & Chandra S Malnutrition humoral immunity and infection *Indian J Pediatr* 43 159 1976
- 22 Chandra R K Chandra S & Ghai O P Chemotaxis random mobility and mobilization of polymorphonuclear leucocytes in malnutrition *J Clin Pathol* 29 224 1976
- 23 Chandra R K Au H Woodford G & Hyam P Iron status immunocompetence and susceptibility to infection In *Ciba Foundation Symposium on Iron Metabolism* Elsevier Amsterdam 1977 p 249
- 24 Coovadia H M Wesley A Brain P Henderson L G Hallett A R & Vos G H Immunoparesis and outcome in measles *Lancet* i 619 1977
- 25 Ferguson A S Lawlor G J Jr Neumann C G Oh W & Stiehm E R Decreased rosette forming lymphocytes in malnutrition and intrauterine growth retardation *J Pediatr* 85 717 1974
- 26 Greenwood H M Oduloye A J & Stratton D Lymphocyte changes in acute malaria *Trans Roy Soc Trop Med Hyg* 71 408 1978
- 27 Gross R L Reid J V O Newberne P M Burgess H Marston R & Hift W Depressed cell mediated immunity in megaloblastic anemia due to folic acid deficiency *Am J Clin Nutr* 28 225 1975
- 28 Harland P E G Tuberculin reactions in malnourished children *Lancet* i 719 1965
- 29 Higgs J M & Wells R S Chronic mucocutaneous candidiasis Associated abnormalities of iron metabolism *Br J Dermatol Suppl* 8 88 1973
- 30 MacDougall L G Anderson R McNab G M Katz J Immune response in iron-deficient children impaired cellular defense mechanisms with altered humoral components *J Pediatr* 85 833 1975
- 31 Moore D L Heyworth D & Brown J PH induced lymphocyte transformation in leucocyte cultures from malnourished and control Gambian children *Clin Exp Immunol* 17 647 1974
- 32 Naege R L Blanc W & Paul C Effects of prenatal nutrition on the human fetus *Pediatrics* 49 494 1973
- 33 Neumann C O Lawlor C G Stiehm E F Swedseid M E Newton C Herbert J Anwar A J & Jacob M Immunological responses in malnourished children *Am J Clin Nutr* 28 89 1975
- 34 Puffer P R & Serrano C V *Patterns of mortality in childhood* Pan American Health Organization Washington DC 1974
- 35 Scrimshaw N S T aylor C E & Gordon J I *Interactions of nutrition and infection* World Health Organization Geneva 1968
- 36 Selvaraj E J & Bhat K S Metabolic and bactericidal activities of leukocytes in protein-calorie malnutrition *Am J Clin Nutr* 25 166 1972
- 37 Seth V & Chandra R K Opsonic activity phagocytosis and intracellular bactericidal capacity of polymorphs in undernutrition *Arch Dis Child* 47 1972
- 38 Srinisinha S Suskind R Edelman R Charutani C & Olson R E Complement and C3 protein activator levels in children with protein calorie malnutrition *Lancet* i 1016 1973
- 39 Srinisinha S Suskind R Edelman R Asvap C & Olson R E Secretory and serum IgA children with protein calorie malnutrition *Pediatrics* 55 166 1975
- 40 Smythe P M Breton Stiles G G Grace H Mafoyane A Schonland M Coovadia R A Loening W E K Parent M A & Vos G I Thymolymphatic atrophy and depression of cell mediated immunity in protein calorie malnutrition *Lancet* ii 939 1971
- 41 Solberg C O & Hellum A M Neutrophil granulocyte function in bacterial infections *Lancet* ii 7 1977
- 42 Watson E Reyes M A & McMurray D I Influence of malnutrition on the concentration of lysozyme amylase and aminopeptidase in children *Proc Soc Exp Biol Med* 157 215 1978

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BOOK REVIEWS

M Siegel *The clinical management of muscle disease practical manual of diagnosis and treatment* William Heinemann London 1977 161 pp illus £3 74 ISBN 0-316-94351 5

As stated in the preface this monograph has been written as a compendium on the general problems of muscle diseases with the practical aspects of diagnosis and care being stressed. As the author is an orthopaedic surgeon it is natural that the emphasis is directed toward physiatric and orthopaedic therapeutic techniques. The author's personal experience is reflected in the valuable chapters dealing with the postural pathomechanics following weakness and contractures in Duchenne dystrophies as well as the survey of surgical treatment of hip ankle and foot deformities. The difficult problem of scoliosis is also dealt with.

The reader looking for a description of the new congenital myopathies will be disappointed. The section on genetics is not written with the same authority as those about practical treatment. The illustrations consisting of photographic documentation of patients are not of a very high quality. There are numerous spelling errors in the text. In spite of its weaknesses the volume is worth to be read also by members of a habilitation team. The management of orthopaedic problems in muscular dystrophy being to a large extent neglected in neuropaediatric textbooks.

Bo Hellstrom

W Pienert & J Hermann *Anamnen im Kindesalter Reihe Moderne Pädiatrie* VEB Georg Thieme Leipzig 1977 141 pp illus DM 35

For an Anglo-American oriented Swede it was kind of an adventure to read this little book in paperback from GDR. It shows very clearly that paediatric haematology is well developed in Jena where the authors live and work. Further it is obvious that the authors have had access to all pertinent and recent literature even from abroad and by these means and by their own clinical experience and studies they have been able to compile a comprehensive and up-to-date presentation of childhood anaemias. Mostly the story is told in a clear and straight forward way which makes the reading easy even for those who are not very familiar with German. Unnecessary and confusing deviations which used to be common in German scientific publications have been avoided. There are of course statements that could be disputed but they are rare and of minor importance.

Ernst Schaefer

R G Williams & C H Tucker *Echocardiographic diagnosis of congenital heart disease* 352 pp illus Little Brown and Company Boston 1977 \$19 95 ISBN 0-316-94351 7

The use of echocardiography in paediatric cardiology has increased rapidly during the last years and it is therefore natural that handbooks and monographs on this subject are now appearing. The title of the present book is a little misleading since it covers echocardiography in various parts of paediatric cardiology not only congenital heart disease.

The book is based on the experience of the Boston group since 1972. It is thus founded on a large patient series. The book is divided into four parts: the first dealing with the basic technique, the second with normal echocardiography including anatomy, and the third and fourth part discuss the diagnosis of acyanotic and cyanotic heart diseases. Each chapter is presented in a logical way presenting anatomy, examination technique, diagnostic features, divided into definite, suggestive and supporting features, and ending with discussion of pitfalls and differential diagnoses. Each chapter contains several clear illustrative echocardiograms, explaining schematic drawings and a list of pertinent references. As a whole the presentation is made in a very clear didactic way.

My main objection to this presentation concerns the limitations of the ultrasound method. These are in some extent discussed in the sections named pitfalls. It could however have been valuable with an additional discussion about the limitations and pitfalls set by ultrasound physics, such as the problem of lateral resolution, the beam width, the angulation problem with echo dropout etc. The statement that the diagnosis of a ventricular septal defect can be made by echocardiography seems to me dangerous and is certainly a controversial subject.

The main abnormal echocardiographic findings are discussed in a proper way but information of how reliable these signs are would have increased the value of the book.

With such a new technique as echocardiography differences in opinion and interpretation cannot be avoided and must be accepted. The future will increase our experience and tell us more about the reliability of the various abnormal findings. As a whole I think it is important always to interpret the echocardiogram in relation to the other diagnostic information available.

This book can be recommended to all working with echocardiography in paediatric cardiology. One should read it bearing in mind that this is a new technique and that the implication of some echocardiographic abnormalities described needs confirmation in larger materials in order to verify the diagnostic usefulness and particularly the reliability of the method.

Nils Rune Lundström

E. Rossi (ed.) *Moderne Endoscopie im Kindesalter. In Padiatrische Fortbildungskurse für die Praxis* No. 46 122 pp illus S. Karger Basel München Paris London New York Sydney 1977 US\$24.50 ISBN 3 8055 2799 3

John O. Forfar & Gavin C. Arneil (eds.) *Textbook of Paediatrics* 2nd edition Vol 1 and 2 2777 pp illus Churchill Livingstone Edinburgh London and New York 1978 £90.00 ISBN 0-443 01849-0

ANNOUNCEMENTS

XXth CONGRESS OF THE CZECHOSLOVAK PAEDIATRIC SOCIETY

The XXth Congress of the Czechoslovak Paediatric Society with International Participation will take place in Bratislava October 3-5 1979. For further information write to the Congress Office: Slovak Medical Society Michiewiczova 18 88322 Bratislava Czechoslovakia

INTERNATIONAL MEETING ON PATHOPHYSIOLOGY OF PUBERTY

An international meeting on Pathophysiology of Puberty will be held in Bologna Italy June 21-22 1979. For further information please write to Professor Emanuele Cacciani Clinica Pediatrica dell'Università Via Massarenti 40139 Bologna Italy

THE EIGHTEENTH ANNUAL LEO G. RIGLER LECTURE

The 18th Annual Leo G. Rigler Lecture and Convention on Pediatric Radiology—sponsored by the Tel Aviv University Sackler School of Medicine section of diagnostic radiology and the municipal—government medical center Tel Aviv/Yaffo departments of diagnostic radiology is to be held at the Accordia hotel Herzlia on Sea on May 8th to 10th 1979. The Rigler lecturer will be John A. H. putnick Jr. M.D. professor and radiologist in chief Children's Hospital Medical Center Boston Mass. Further information kindly contact professor S. Schichilov Hospital Tel Aviv Israel.

THE INTERNATIONAL WORKSHOP ON THE AT RISK INFANT

The International Workshop on the At Risk Infant will take place in Tel Aviv (Israel) July 25-27 30-31 1979 the International Year of the Child. For further information write to the secretary Shaul Harel M.D. P.O. 16271 Tel Aviv Israel.

BOOK REVIEWS

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This book can be recommended to all working with echocardiography in paediatric cardiology. One should read it bearing in mind that this is a new technique and that the implication of some echocardiographic abnormalities described needs confirmation in larger materials in order to verify the diagnostic usefulness and particularly the reliability of the method.

Nils Rune Lundström

ACKNOWLEDGEMENT

The Editorial Board of *Acta Paediatrica Scandinavica* wishes to express its sincere gratitude to the following persons outside the Advisory Board who

have acted as referees during the past year. The standard of the journal depends to a very large extent on the skill and interest of these reviewers

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LONG TERM PROGNOSIS OF INFANTS WITH IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

Follow up Studies in Infants Surviving after the Introduction of Continuous Positive Airway Pressure

JENS KAMPER and JÖRN MÖLLER

From the Department of Neonatology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

ABSTRACT Kamper J & Möller J (Department of Neonatology, Rigshospitalet, University of Copenhagen, Denmark). Long term prognosis of infants with idiopathic respiratory distress syndrome. Follow up studies in infants after the introduction of Continuous Positive Airway Pressure. *Acta Paediatr Scand* 68 149 1979. — Fifty one children surviving IRDS with CPAP alone or CPAP and IPPV were studied at the age of 2.5 to 4.0 years. One child had developed tetraplegia and mental retardation and 6 children were speech retarded. Correlation with perinatal events showed that this group of children had a significantly lower gestational age and birth weight, a lower Apgar score and a higher $P_{\text{a}}\text{CO}_2$ prior to ventilatory treatment than the remainder. Re-examination by age 4.0 to 5.0 years showed persistent handicaps in only four of the seven children.

KEY WORDS Newborn infants, respiratory distress syndrome, artificial respiration, neurological findings.

Ventilatory support of infants with idiopathic respiratory distress syndrome (IRDS) is today a well established procedure. In a relatively short span of years technique and methods have been developed which have improved the early prognosis of this serious condition remarkably. A particularly important milestone was the introduction of the continuous positive airway pressure (CPAP) (10-24) which significantly simplified the treatment, lessening the risks of dangerous complications. Publications dealing with the long term prognosis from this period are scarce (1). In the following we therefore will present our follow up results in 51 children who survived IRDS after CPAP had begun to be used.

MATERIALS AND METHODS

The neonatal department of Rigshospitalet has employed mechanical ventilation since 1966 (2). CPAP was adopted in August 1971 and during the following 70 months a total of 94 infants with IRDS received ventilatory support either with CPAP, intermittent positive pressure ventila-

tion (IPPV) or both (Fig. 1). Fifty five infants survived (58.5%) but 4 were lost to follow up, leaving a total of 51 survivors to participate.

IRDS was diagnosed when the infants developed progressive respiratory failure with tachypnoea, grunting, recessions and increasing oxygen dependency and the characteristic chest X-ray configuration could be demonstrated (3,4). Neonatal and therapeutic characteristics of the 51 survivors are indicated in Table 1. Twenty nine (57%) infants were born after pregnancies complicated by bleeding episodes, pre-eclampsia, placental insufficiency, rhesus immunization or maternal diabetes mellitus and 21 (41%) were delivered by caesarean section or in irregular presentations. Seven infants had an Apgar score equal to 3 or lower after 5 minutes or did not cry during this period. Six infants had a birth weight lower than 1501 g and 6 infants were small for dates, having birth weights below the 10th centile (3). Twenty four infants (47%) were born in other hospitals and transferred as emergency cases. Thirty were boys and 21 girls.

Umbilical artery catheters were inserted when the infants required more than 40% oxygen for the purpose of following the arterial $P_{\text{a}}\text{O}_2$ and acid-base status and to provide a parenteral route for fluid administration. Infants admitted in gross respiratory failure were not catheterized till after the ventilatory support had been initiated, however. In 6 survivors the arterial catheterization failed. Whenever umbilical arterial catheters were not available oxygen saturation and acid-base parameters were obtained on blood drawn by heel sticks. The $P_{\text{a}}\text{O}_2$ and acid

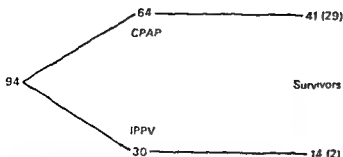


Fig. 1 94 infants distributed according to primary mode of ventilatory support. Infants surviving with only one mode of ventilatory support are indicated in parentheses

base analyses were performed using the Radiometer BMS II

Parenteral fluid therapy was started on the first day of life and gastric tube feeding with mother's milk or milk substitute (Nan[®]) was initiated on the second. Accurate information concerning milk intake could be obtained in 44 infants who received an average of 502 ml milk per kilo birth weight during the first week of life. Records of body weight at the end of the first week were available in 31 infants.

CPAP was administered *ad modum* Gregory et al. (10). As shown in Fig. 1 a total of 64 infants could be treated initially with CPAP indicated by P_i values below 6.66–7.98 kPa (50–60 mm) and/or general cyanosis combined with hypercapnia and acidosis in spite of breathing pure oxygen through a close fitting face mask. These conditions had previously been chosen as indications of ventilator therapy in our department (2, 14).

CIAP was mostly administered via naso tracheal tube (18). The so-called Gregory box (10) was tried out in 8 infants 3 of whom survived. The box was abandoned because of difficulties with the neck seal which caused ulcerations in 2 cases.

Twenty nine out of 64 infants survived with CPAP only while 12 survivors also received IPPV because of progressive respiratory insufficiency in spite of 12–14 cm CPAP and 100% oxygen. Eighteen survivors received treatment with CPAP because of a low P_{aO_2} (mean 5.72) and 18 because of combined general cyanosis, hypercapnia (mean P_{iO_2} 7.85 kPa) and acidosis (mean pH 7.23).

As indicated in Fig. 1 23 infants died—2 during CPAP treatment the remainder while on ventilator. Autopsies showed massive intracranial haemorrhage in 13 and pulmonary haemorrhage in 1, bronchopneumonia in 2, isolated pulmonary fibroplasia in 1 infant. In 4 cases death was ascribed to intractable atelectasis.

Mean birth weight was relatively high in infants surviving with CPAP only (2320 ± 660 g) when compared with survivors who also needed IPPV (1896 ± 285 g, $p < 0.01$) and all infants needing IPPV later (1798 ± 681 g, $p < 0.01$). No significant differences between the above mentioned groups were found in mean values of gestational age (P_{aO_2} , pH and P_{CO_2} prior to or in age at initiation of CPAP).

Ventilator treatment

Ventilatory techniques and methods used have been published previously (2). The indication for ventilator treatment in these series was general cyanosis and highly insufficient respiratory movements (often apnoea) in spite of 100% inspiratory oxygen. Thirty infants were ventilated but only 14 survived (Fig. 1).

Preventilatory acid-base values were obtained in 6 survivors (mean pH 7.15, mean P_{CO_2} 10.37 kPa). Twelve of 14 survivors were weaned off the respirator using CPAP.

Autopsy performed in all fatal cases except one showed intracranial haemorrhage in 6, significant diffuse pulmonary haemorrhage in 1 and bronchopneumonia in 1 infant. In the remainder death was ascribed to massive diffuse atelectasis.

Ophthalmoscopic examinations

These were performed on the ward at regular intervals and repeated in case of suspect findings and in infants with birth weight below 2000 g when at least 2 months old.

Follow up procedure

Forty four survivors were examined at our follow up clinic while 6 had to be examined at home. One of these who had emigrated was examined by a local physician. Another child who also lives abroad participated by questionnaire only. With these two exceptions all children were examined by one of the authors (J. M.). Four survivors did not participate in the follow up but 2 of these were judged normal at routine controls by age 3 years.

Table 1 Neonatal and therapeutical characteristics of 51 infants surviving with ventilator support

	Range	Mean	(Mean)
Birth weight (g)	1 250–3 550	2 197	(2 446)
Gestational age (weeks)	28–39	34.2	(34.8)
Age ventilatory support begun (h)	4–55	25	(35)
Duration of ventilatory support (h)	3–633	101	(144)
Duration of oxygen treatment $\geq 70\%$ (h)	3–355	72	(211)
Duration of oxygen treatment any conc. (h)	67–1 800	763	(425)

Mean values are indicated in parentheses for 75 infants surviving IRDS with the aid of IPPV from the preceding 5 year period.

Table 2 Result of neurological and psychological examination in 7 IRDS survivors with doubtful or certain handicaps at first follow up

Patient	First follow up			Last follow up		
	Age (yr)	Linguistic test	DDST	Age (yr)	Leiter performance test (IQ)	Binet intelligence test (IQ)
VM	9	Abnormal	Abnormal	4.6	91	-
TJN	3.75	Abnormal	Normal	4.0	98	-
CH	7.5	Abnormal	Normal	4.5	104	77
FM	3.3	Abnormal	Normal	5.0	107	90
LAC	7.5	Abnormal	Normal	4.0	111	97
PSL	7.5	Abnormal	Normal	4.1	116	100
BN	5	Abnormal	Normal	4.0	116	108
						Major neurological and psychological findings
						Tetraplegia
						Dysphasia epilepsy
						Minimal brain dysfunction
						Dysphasia
						Dysphasia?
						0
						0

The age at follow up ranged from 3.5 to 4 years with a mean of 3.

A questionnaire was forwarded to the parents to allow them to complete in advance anamnestic details of the child and for comparison the next younger or older sibling if any. On the day of the follow up the questionnaires were scrutinized. The children were given a physical and neurological examination and height, weight and head circumference were measured. Fifty children were evaluated with the revised Denver developmental screening test (DDST) (8-9) which has proven both sensitive and specific with a high degree of agreement with more comprehensive developmental and intelligence tests. Vocabulary and linguistics were evaluated further with a Danish linguistic test (5) which is standardized for children between 3.0 and 6.5 years. Reference values for children below 3.0 years of age were calculated by extrapolation. Seven children who presented with major neurological abnormalities or abnormal linguistic performance were admitted to paediatric neurologists or psychologists for more detailed evaluation which usually included Binet's intelligence test (Danish revision 1943) and always a Leiter's performance test.

Children seen at Rigshospitalet had chest X-rays taken in the antero-posterior and lateral projections. The radiographs were evaluated blindly by 2 radiologists (H.S. and L.J.).

RESULTS

Growth

Compared to values predicted from recent Swedish growth curves (6) the mean weight of the survivors was 0.4 kg below mean for age while mean height did not differ. The mean head circumference was 1.0 cm above mean. No child had a head circumference larger than ± 2 S.D. of the curves however and none was suspected of developing hydrocephalus.

Development

Early motor development was estimated from parental dating of the first step taken without support. One child who had developed a tetraplegia did not walk by the age of 2.75 years. The remainder walked on an average by age 14.4 months. Thirty-two survivors were compared with their siblings. The ability to walk alone was found on an average to be retarded by 1.9 months ($p < 0.01$).

As to speech development 40% of the IRDS survivors had developed considerably more slowly than their siblings which is significant ($\chi^2 = 9.4$, $p < 0.01$).

Neurological and psychological examination

A total of 7 IRDS survivors presented with doubtful or certain handicaps by age 2.5 to 4 years as indicated in Table 2. Only 4 of these had remaining moderate to severe handicaps when re-evaluated at the age of 4 to 5 years; however, Strabismus which was found in 10 children and febrile convulsions occurring in 4 were not considered handicaps. None developed retrolental fibroplasia.

The 7 IRDS survivors with abnormal linguistic performance including the child with cerebral palsy were compared with the remainder with regard to a number of neonatal parameters employing Student's *t*-test as shown in Table 3. The statistical analysis showed that this group of children was not

Table 3 Neonatal and therapeutic parameters in 43 children with normal and 7 with abnormal linguistic performance (Bo Ege Linguistic Test)

NS=Not Significant

	Normal		Abnormal		Probability
	N	Mean \pm 1 S D	N	Mean \pm 1 S D	
Gestational age (weeks)	43	34.0 \pm 2.4	7	31.8 \pm 2.5	<0.05
Birth weight (g)	43	2 277 \pm 600	7	1 734 \pm 193	<0.05
Apgar score 1 min	40	7.5 \pm 2.5	7	4.2 \pm 1.9	<0.005
Apgar score 5 min	37	8.9 \pm 1.5	7	7.8 \pm 1.4	NS
P _{ao2} before ventilatory support (kPa)	34	8.1 \pm 1.9	5	10.9 \pm 3.2	<0.01
pH before ventilatory support	34	7.21 \pm 0.09	5	7.12 \pm 0.06	NS
Age ventilatory support begun (h)	43	26 \pm 16	7	18 \pm 17	NS
Duration of ventilatory support (h)	43	94 \pm 97	7	152 \pm 153	NS
Intake of milk (ml/kg birth weight/l wk)	36	495 \pm 213	7	517 \pm 154	NS
Weight loss (g/kg birth weight/l wk)	35	79 \pm 44	6	87 \pm 36	NS

only more asphyctic at delivery, smaller and more premature but also more asphyctic prior to ventilatory treatment than the other group. The mode of ventilatory treatment was of no prognostic significance, e.g. the child with cerebral palsy survived with CPAP only.

Cardio pulmonary examination

Ten IRDS survivors had been admitted to hospital at least once because of lower respiratory tract infections. No children suffered from laryngeal stenosis and none presented with dyspnoea or cyanosis. One child presented with a patent ductus arteriosus which was successfully closed 6 months later.

The X-ray examination revealed minor changes in 10 IRDS survivors, the predominant abnormality being discrete parenchymal line shadows. None had emphysema or cor pulmonale.

DISCUSSION

During the last decade ventilatory support of newborn infants with respiratory insufficiency, mainly IRDS, has come into common use. Controlled studies have reported increased survival rates with ventilator treatment (19-22) and a number of follow-up studies has encouraged the development further in that the majority of these high risk infants

has been shown to develop normally (4, 7, 12, 13, 14, 17, 20, 23). These studies have all shown that some survivors may develop significant handicaps, e.g. cerebral palsy, hydrocephalus, intellectual deficiency and retrolental as well as pulmonary fibroplasia. Results from different centres are difficult to compare due to differences in composition of material, i.e. number of referrals and very small prematures, and probably in a large number of therapeutic details. Comparisons from period to period within the same centre seem more adequate, although it must be emphasized that various improvements in nursery routines, monitoring etc. may influence the outcome. Compared with the preceding 5-year period (14), the survival rate in infants ventilated for IRDS at our centre increased from 38.6 to 58.5% (and later to nearly 80%) (15). At the same time the prevalence of neurological and mental sequelae decreased from 17.8% and the type and extent of handicaps became more moderate. As an increased number of small prematures has survived (Table 2), these changes strongly suggest that a considerable improvement in the outlook of our ventilated IRDS survivors has taken place. Most survivors in this study have been treated exclusively or partially with CPAP, which is thought to have both modified the ventilator treatment as a whole, as indicated in Table

and contributed significantly to improvements in both short (15) and long term prognosis

Although only 4 survivors displayed lasting handicaps more infants are thought to have suffered from cerebral depression during the perinatal period as 32 survivors who could be compared with siblings seemed retarded as to their early motor and speech development. Furthermore 7 survivors at the time of the first follow up were classified as speech retarded at the linguistic test which is 3 or 4 more than predicted. This early speech retardation was significantly related not only to low birth weight and gestational age but also to low Apgar score at 1 minute and hypercapnia prior to ventilatory support. Similarly Hof & Weissner (12) found a significant relation between retarded psychomotor development and pre-ventilatory hypercapnia. The possible risk of early hyponutrition has been stressed by Stahlman and co workers (23) who speculated whether psychological deficiencies in childhood could be ascribed to neonatal starvation during mechanical ventilation. Our previous follow up study (14) did in fact give support to this theory as survivors with psychomotor or mental retardation had been supplied with significantly less milk per kg birth weight than the remainder during the first week of life. In contrast speech retardation in the present series was not related to low milk intake (or weight loss) during the first week. This may be due to the fact that the average milk intake has increased considerably i.e. from 305 to 502 ml/kg birth weight.

Thus further improvements in long term prognosis given adequate nutrition may depend largely on prevention of pre-ventilatory asphyxia for instance by increased use of car diotachographia and by earlier initiation of ventilatory support (16).

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REFERENCES

- 1 Ahlstrom H, Jonson B & Svenningsen N W. Continuous positive airways pressure treatment by a face chamber in idiopathic respiratory distress syndrome. *Arch Dis Child* 51 13 1976.
- 2 Cooke R, Lundmark M, Lomholt N F, Yssing M, Zachau-Christiansen B & Fris-Hansen H. Respiratory failure in the newborn. The techniques and results of intermittent positive pressure ventilation. *Acta Paediatr Scand* 56 498 1967.
- 3 Davies P A, Robinson R J, Scopes J W, Tizard J P M & Wigglesworth J S. Birth weight/gestational age charts. In *Clinics in developmental medicine* nos 44/45. Medical care of newborn babies. Spastics International Medical Publications London 1977. W. Heinemann Medical Books Ltd Oxford. J B Lippincott Co Philadelphia p 373.
- 4 Dinwiddie R, Mellor D H, Donaldson S H C, Tunstall M E & Russell F. Quality of survival after artificial ventilation of the newborn. *Arch Dis Child* 49 703 1974.
- 5 Ege B. Sprogtest I. Sprogaldertest. *Tale og Stemme* 1 21 1969.
- 6 Engstrom I, Karlberg P, Klackenborg G, Klackenborg Larsson I, Lichtenstein H, Svennberg I & Taranger J. Growth diagrams for height, weight and head circumference from birth to the age of 16 years. *Läkartidningen* 70 7952 1973.
- 7 Fitzhardinge P M, Pape K, Arstikaitis M, Boyle M, Ashby S, Rowley A, Netley C & Swyer F R. Mechanical ventilation of infants of less than 1501 gm birth weight. Health, growth and neurological sequelae. *J Pediatr* 88 531 1976.
- 8 Frankenburg W K & Dodds J B. The Denver developmental screening test. *J Pediatr* 71 181 1967.
- 9 Frankenburg W K, Goldstein A D & Camp B W. The revised Denver developmental screening test: its accuracy as a screening instrument. *J Pediatr* 79 988 1971.
- 10 Gregory G A, Kitterman J A, Plubbs R H, Tooley W H & Hamilton W K. Treatment of the idiopathic respiratory distress syndrome with continuous positive airway pressure. *N Engl J Med* 284 1333 1971.
- 11 Harrod J R, L'Heureux M, Wangenstein O D & Hunt C E. Long term follow up of severe respiratory distress syndrome treated with IPPB. *J Pediatr* 84 277 1974.
- 12 Hof M & Weissner K. Spätsfolgen nach intermittierender Überdruckbeatmung bei schwerem idiopathischem respiratorischen distress syndrome. *Schweiz Med Wochenschr* 99 1853 1969.
- 13 Johnson J F, Malachowski N C, Grobstein R, Welsh D, Daily W J R & Sunshine H. Prognosis of children surviving with the aid of mechanical ventilation in the newborn period. *J Pediatr* 84 277 1974.
- 14 Kamper J. Long term prognosis of infants with severe idiopathic respiratory distress syndrome. I. Neurological and mental outcome. *Acta Paediatr Scand* 67 61 1978.

- 15 Kamper J, Bækgaard P, Petersen B, Mørsting Christensen P, Tygstrup I & Friis Hansen B. Respiratory distress syndrome of the newborn treated with continuous positive airway pressure. *Ugeskr Læk* 135 2207 1973
- 16 Krouskopp R W, Brown F G & Sweet A V. The early use of continuous positive airway pressure in the treatment of idiopathic respiratory distress syndrome. *J Pediatr* 87 263 1975
- 17 Marriage K J & Davies P A. Neurological sequelae in children surviving mechanical ventilation in the neonatal period. *Arch Dis Child* 52 176 1977
- 18 Mørsting Christensen P, Kamper J, Petersen B, Bækgaard P & Friis Hansen B. Technique and method of treatment of respiratory distress syndrome with continuous positive airway pressure (CPAP). *Ugeskr Læk* 135 2212 1973
- 19 Murdoch A I, Limb J, McC Reid M, Sutton M D, Tilik A S, Ulin O A & Swyer P R. Mechanical ventilation in the respiratory distress syndrome. A controlled trial. *Arch Dis Child* 45 624 1970
- 20 Outerbridge F W, Ramsay M & Stern L. Developmental follow up of survivors of neonatal respiratory failure. *Crit Care Med* 2 23 1974
- 21 Peterson H G & Pendleton M E. Contrary roentgenographic patterns of the hyaline membrane and the fetal aspiration syndromes. *Am J Roentol* 74 800 1955
- 22 Reid D H S, Tunstall M E & Mitchell R G. Controlled trial of artificial respiration in the respiratory distress syndrome of the newborn. *Lancet* ii 537 1967
- 23 Stahlman M, Hedwall G, Dolanski E, Faxälv G, Burko H & Kirk A. A six year follow up of clinical hyaline membrane disease. *Pediatr Clin N Am* 20 433 1973
- 24 Wung J T, Stark R L, Hegyi T, Driscoll J & James L S. CDP. A major breakthrough. *Pediatrics* 58 783 1976

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A CLINICAL AND NEUROPHYSIOLOGICAL INVESTIGATION OF A DANISH KINDRED WITH HETEROZYGOUS FAMILIAL HYPOBETALIPOPROTEINEMIA

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ABSTRACT Andersen G E Trojaborg W and Lou H C (Neonatal Department and Department of Neurophysiology Rigshospitalet Copenhagen Denmark) A clinical and neurophysiological investigation of a Danish kindred with heterozygous familial hypobetalipoproteinemia. *Acta Paediatr Scand* 68 155 1979.—A three-generation transmission of under five percentile values for serum low density lipoprotein and low density lipoprotein cholesterol typical of heterozygous familial hypobetalipoproteinemia was demonstrated in a Danish family. Slight clinical signs of CNS abnormality were found in 4 of the 8 subjects with heterozygous familial hypobetalipoproteinemia but did not resemble the neurological findings in abetalipoproteinemia nor in the previously described patients with familial hypobetalipoproteinemia. There were no signs of myelin dysfunction in the central nervous system as judged from the normal latency of visual and somatosensory evoked potentials.

KEY WORDS Cholesterol triglyceride lipoproteins genetics myelin EEG cortical evoked potentials

Familial hypobetalipoproteinemia (FHBL) is a genetic disorder which differs from classical abetalipoproteinemia (ABL) by having an abnormally low yet immunochemically identifiable serum LDL in the proband and at least one first degree relative in the absence of diseases to which hypobetalipoproteinemia may be secondary (trauma infections fat malabsorption hyperthyroidism hepatic necrosis severe anemia anti apo B myeloma protein production). An autosomal dominant mode of inheritance is most likely (1). FHBL has been described in a heterozygous and homozygous form. The heterozygous condition is normally asymptomatic there is however data which suggests that the low serum LDL C levels may deter the development of coronary heart disease leading to a longer than average life expectancy (2). Homozygotes are biochemically indistinguishable from patients with ABL having no apolipoprotein B nor LDL but do not seem to have the same serious central and peripheral neuromuscular

degeneration typical of ABL (3 4 5 6). The cause of the low serum LDL concentration is obscure. Levy et al (7) studied the metabolism of radioiodinated LDL and found a 3 fold lower synthetic rate of LDL in FHBL heterozygotes compared to normals. Furthermore Sigurdsson et al (8) have found a decreased synthetic rate of VLDL apo B and LDL apo B in heterozygotes. Serum LDL is supposed to be a vehicle for substances such as cholesterol linoleic acid vitamin A and E probably all essential for the metabolism of myelin (9 10 11). Therefore in FHBL subjects with very low levels of serum LDL signs of impaired myelination might be expected. In 6 of the 57 heterozygotes so far recorded have

Abbreviations

FHBL=familial hypobetalipoproteinemia ABL=abetalipoproteinemia TC=total cholesterol VLDL-C=very low density lipoprotein cholesterol LDL-C=low density lipoprotein cholesterol HDL-C=high density lipoprotein cholesterol TG=triglyceride VEP=visual evoked potentials SEP=somatosensory evoked potentials

Table 1 Serum lipids and lipoproteins in the individual subjects

Kindred	Age (years)	T C (mmol/l)	VLDL C (mmol/l)	VLDL (EID) (mg/100 ml)	LDL C (mmol/l)	LDL (EID) (mg/100 ml)
I 2	75	3.58	0.52	44	1.67	118
II 1	46	3.47	0.47	42	1.74	137
III 1	19	2.87	0.21	18	0.70	171
III 2	17	3.24	0.75	23	0.84	119
III 3	14	4.34	0.06	5	1.78	137
III 4	12	3.94	0.15	15	1.11	110
III 5	10	5.22	0.12	16	2.70	496
III 6	8	3.53	0.08	8	1.31	161
III 7	7	3.97	0.08	0	1.18	44
III 8	5	4.34	0.12	12	1.63	450
III 9	3	4.54	0.04	8	2.63	687
III 10	1 ⁷ / ₁₂	3.17	0.20	10	1.33	274
III 11	At birth	1.65	0.06	-	0.39	-

neurologic symptoms been described (12-13, 14-15). In 3 of the 6 however, it is not even certain that the neuromuscular abnormalities were related to low levels of serum LDL.

Since the disorder has not earlier been re-

corded in Scandinavia and since the neurological symptoms in FHBL are still very scant and inconsistent we here present the clinical and neurophysiological findings in a Danish family with heterozygous FHBL.

Table 2 Neurological and neurophysiological findings in the individual subjects

Kindred	Age (years)	Clinical findings	EEG	SEP and VEP
I 2	75	Babinski sign bilaterally Impaired vibration sense (absent on lower extremities)	Normal	Normal
II 1	46	Babinski sign (right) Slight finger dysidiadochokinesia	Normal	Normal
III 1	19	None	Normal	Normal
III 2	17	Atypical plantar reflexes Increased extensor tone Slight choreoathetosis Slight constructional dyspraxia	3-4 Hz activity predominantly in temporal regions	Normal
III 3	14	None	Normal	Normal
III 4	12	None	Normal	Normal
III 5	10	None	At rest normal Generalized 4-5 Hz paroxysmal activity with spikes evoked by photic stimulation	Normal latency Amplitudes 175% increased at stimulation of both median nerves
III 6	8	Prominent associated movements	Normal	Normal
III 7	7	Increased extensor tone Prominent associated movements Choreoathetosis and finger dyskinesia Slight constructional dyspraxia	Focal spike activity in left temporal region Normal background activity	Normal
III 8	5	None	Normal	Normal
III 9	3	None	Normal	Normal
III 10	1 ⁷ / ₁₂	None	Normal	Normal
III 11	At birth	None	Normal	-

LDL C (mmol/l)	TG (mmol/l)	Diagnoses
39	1.40	FHBL
5	1.73	FHBL
96	0.73	FHBL
15	0.79	FHBL
50	0.51	Normal
68	0.71	FHBL
40	0.77	Normal
14	0.65	FHBL
71	0.71	FHBL
19	0.88	Normal
87	0.73	Normal
184	0.89	FHBL
10	0.10	Normal

MATERIAL

During our screening of 10000 Danish newborns for hyper and hypolipoproteinemia in February 1976 we identified a newborn girl with a very low level of cord serum VLDL+LDL (<4th percentile value for normal Danish newborns (16)). She was born as number 10 of III children. Pregnancy and delivery was uneventful. No drugs had been given and there were no signs of perinatal asphyxia. She was born in the 39th week of gestation and weighed 3950 g. Apgar score was 10/1 and 5 min. The neonatal and subsequent development has been normal. All members of her family were investigated and she was herself reinvestigated at age 17 years. Meanwhile child number 11 also a girl was born. Pregnancy and delivery was normal. Her cord serum lipids were also determined.

METHODS

Mixed arterial and venous cord blood was obtained after clamping and cutting the umbilical cord within the first 3 min after birth and prior to delivery of the placenta. The cord blood was stored at 4°C for no longer than 17 hours before serum was separated (000 r p.m. 0 min) and the analyses begun. Venous blood samples were taken from the family members after a 10-1 hour fast during a period of stable weight and food habits. The serum was separated and stored at 4°C and the analyses begun within 4 hours. Serum T-C TG VLDL-C LDL-C were measured by the methods described earlier (17). Lipoproteins were separated in a 40.3 rotor Beckman Type L ultracentrifuge at 10°C at 40000 r p.m. for 0- hours. Tube slicing was used as described earlier (17). Furthermore VLDL and LDL was measured by electroimmunodiffusion (EID) using an antiserum raised against apo B as described by Andersen & Gry-Nielsen (17).

The clinical and neurophysiological examination was made by H. E. L. and W. T. without prior knowledge of the individual's serum lipid and lipoprotein values. At the time of the examination none of the subjects had been

informed of the diagnosis nor were any of them aware of or disabled by neurological symptoms.

Visual potentials (VEP) were evoked by flash or pattern reversal and recorded between electrodes placed over the occipital lobes with a reference electrode placed 2 cm in front of vertex during bilateral monocular stimulation as described by Dawson (18-19). Cobb & Morton (20) and Halliday *et al.* (21). Somatosensory potentials (SEP) were evoked by stimulating the median nerve at wrist and recorded between an electrode placed over the hand area of the contralateral sensory cortex and one 2 cm in front of vertex as described by Dawson (18-19) and Giblin (22). The leads were connected to EMG amplifiers (DISA 15Col) with a lower limiting frequency of 0.5 Hz and an upper limiting frequency of 1000 Hz (3 dB down). The evoked cortical potentials were recorded on line by electronic averaging of 1-8-56 responses using a 4 channel signal averager (Nicolet 1074) and was displayed via a X-Y writer (HP 7044 A).

RESULTS

The kindred is presented in Fig. 1 and the individual serum lipids, diagnoses, clinical and neurophysiological findings are given in Tables 1 and 2. Three of the children with FHBL (III 2, III 4 and III 7) deserve special attention as they have additional symptoms to the ones outlined in Table 2.

III 2 is a 17-year-old girl who for the last three months before the study experienced or thostatic dizziness, the cause of which seems to be intermittent hypertension, systolic blood pressures being 120-160 and diastolic blood pressures between 80-100 mmHg. There were no signs of retinopathy nor cardiac ventricular hypertrophy. Renal and adrenal causes of hypertension were ruled out, as was coarctation of the aorta. Glucose tolerance test was normal. Minor neurological and neurophysiological abnormalities were found as described in Table 2.

III 4 is a 12-year-old boy with diabetes mel



Fig. 1 The kindred

Table 1 Serum lipids and lipoproteins in the individual subjects

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I 2	75	3.99	0.52	44	1.67	318
II 1	46	3.47	0.47	42	1.75	333
III 1	19	2.87	0.21	18	0.70	171
III 2	17	3.24	0.25	23	0.84	179
III 3	14	4.34	0.06	3	1.78	337
III 4	12	3.94	0.15	15	1.11	229
III 5	10	5.22	0.17	16	2.70	496
III 6	8	3.53	0.08	8	1.31	61
III 7	7	3.97	0.09	0	1.18	244
III 8	5	4.34	0.12	12	2.63	480
III 9	3	4.54	0.04	8	2.63	687
III 10	1 ^{1/2}	3.37	0.70	20	1.33	274
III 11	At birth	1.65	0.06	-	0.19	-

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III 3	14	None	Normal	Normal
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III 5	10	None	At rest normal Generalized 4-5 Hz paroxysmal activity with spikes evoked by photic stimulation	Normal latency Amplitudes 125% increased at stimulation of both median nerves
III 6	8	Prominent associated movements	Normal	Normal
III 7	7	Increased extensor tone Prominent associated movements Choreoathetosis and finger dyskinesia Slight constructional dyspraxia	Focal spike activity in left temporal region Normal background activity	Normal
III 8	5	None	Normal	Normal
III 9	3	None	Normal	Normal
III 10	1 ^{1/2}	None	Normal	Normal
III 11	At birth	None	Normal	Normal

- Cottrill C Glueck C J Leuba V Millet F Puppione D & Brown W V Familial homozygous hypobetalipoproteinemia *Metabolism* 23 779 1974
- Salt H B Wolff O H Lloyd J K Forbrooke A S Cameron A H & Hubble D V On having no beta lipoprotein A syndrome comprising abetalipoproteinemia acanthocytosis and steatorrhea *Lancet* II 3 5 1960
- Muller D P W & Lloyd J K The long term management of abetalipoproteinemia a possible role for vitamin E *Arch Dis Child* 52 09 1977
- Biemer J J & McCammon R E The genetic relationship of abetalipoproteinemia and hypobetalipoproteinemia A report of the occurrence of both diseases within the same family *J Lab Clin Med* 85 556 1975
- Levy R I Langer T Gotto A M & Fredrickson D S Familial hypobetalipoproteinemia a defect in lipoprotein synthesis *Clin Res* 18 539 1970
- Sigurdsson G Nicoll A & Lewis H Turnover of apolipoprotein B in two subjects with familial hypobetalipoproteinemia *Metabolism* 26 5 1977
- Isvetbacher K J Scheig R Plotzkin G R & Caulfield J B Congenital beta lipoprotein deficiency An hereditary disorder involving a defect in the absorption and transport of lipids *Medicine (Baltimore)* 43 347 1964
- Fredrickson D S Levy R I & Lees R S Fat transport in lipoproteins An integrated approach to mechanisms and disorders *N Engl J Med* 276 96 1967
- Lees R S & Ahrens E H Fat transport in abetalipoproteinemia The effects of repeated infusions of beta lipoprotein rich plasma *N Engl J Med* 280 1 61 1969
- Mars H Lewis L A Robertson A L Butkus A & Williams G H Familial hypo-beta lipoproteinemia A genetic disorder of lipid metabolism with nervous system involvement *Am J Med* 46 886 1969
- Brown H J Lewis L A & Mercer R D Familial hypobetalipoproteinemia Report of a case with psychomotor retardation *Pediatrics* 54 111 1974
- Tamir I Levitov O Lotan D Lequin C Heltenberg D & Werbin B Further observations on familial hypobetalipoproteinemia *Clin Genet* 9 149 1976
- Mawatan S Iwashita H & Kuroiwa Y Familial hypo-beta lipoproteinemia *J Neurol Sci* 16 93 1972
- Andersen G E Lous P & Friis Hansen B Screening for hyper and hypolipoproteinemia in 10000 Danish newborns *Acta Paediatr Scand* in press
- Andersen G E & Gry Nelsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL LDL *Clin Chim Acta* 66 79 1976
- Dawson G H Cerebral responses to electrical stimulation of peripheral nerve in man *J Neurol Neurosurg Psychiatr* 10 134 1947
- Dawson G D A summation technique for the detection of small evoked potentials *Electroencephalogr Clin Neurophysiol* 6 65 1964
- Cobb W A & Morton H H The human electroretinogram in response to high intensity flashes *Electroencephalogr Clin Neurophysiol* 4 547 1957
- Halliday A M McDonald W I & Mushin J Delayed visual evoked response in optic neuritis *Lancet* I 987 1977
- Giblin D R Somatosensory evoked potentials in healthy subjects and in patients with lesions of the nervous system *Ann NY Acad Sci* 112 97 1964

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litus since the age of 4. Initially he was treated with protamine zinc insulin (Retard Leo[®]) 20 IU in the morning and 1600 kcal/day. Over the years he has experienced hypoglycemic episodes demanding minor adjustments of the amount of insulin and calories. He is at present well regulated on zinc insulin (Monotard Novo[®]) 18 IU + Semi Lente (Novo[®]) 10 IU in the morning and 2200 kcal/day. His growth and psychomotor development have been normal. Neither neurological nor neurophysiological abnormalities were found.

III 7: a 7-year-old girl with diabetes mellitus since the age of 6 was initially treated with protamine zinc insulin (Retard Leo[®]) 6 IU in the morning and 1690 kcal/day. She has had diabetic acidosis twice in connection with otitis media. After adenoidectomy she is now well regulated on protamine zinc insulin (Retard Leo[®]) 18 IU + regular insulin (Neutral Leo[®]) 2 IU in the morning and 2000 kcal/day. Her growth and psychic development have been normal. Minor neurological abnormalities were found and her EEG was severely abnormal as described in Table 2.

DISCUSSION

In ABL subjects with a total lack of serum apolipoprotein B and LDL the clinical symptoms and signs suggest a combination of neuronal and myelin sheath degeneration. In FHBL subjects with very low levels of serum apolipoprotein B and LDL one might therefore expect minor signs of myelin dysfunction. Thus far 57 individuals have been reported to have FHBL. In only three cases however have neurological abnormalities been described.

In a 37-year-old woman Mars et al (12) found clumsiness, dysesthesia of lower extremities, general weakness and hyperactive deep tendon reflexes, Babinski sign and ataxia. During 1–2 years her condition deteriorated with relapses suggestive of multiple sclerosis. Tarrar et al (14) found FHBL in a 4-month-old boy with microcephaly and infantile

spasms. Brown et al (13) found FHBL in a 6-month-old boy with slow psychomotor development but without seizures.

In the present kindred four of the eight individuals with FHBL had slight–moderate clinical signs of CNS abnormality. The 75-year-old grandmother (I 2) and her 46-year-old son (II 1) had abnormal plantar responses suggesting minimal dysfunction of the corticospinal tracts. In the two girls (III 2 and III 7) the findings suggest involvement of both basal ganglia and the corticospinal tracts. These findings, however, are not similar to the severe neurological symptoms resembling Friedreich's ataxia in ABL, nor can they be related to the neurological findings in the three patients with FHBL previously mentioned.

There were no signs of myelin dysfunction in the CNS in any of the eight subjects with FHBL as judged by the normal latency of visual and somatosensory evoked potentials.

We therefore conclude that the clinical neurological findings in the present and earlier described individuals with FHBL are either an expression of an exceptional clinical variability or coincidental. It is difficult to attribute the findings to one nosological entity, i.e. FHBL. Finally the neurophysiological data seem to exclude CNS myelin dysfunction in the FHBL subjects here described.

ACKNOWLEDGEMENT

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REFERENCES

- Herbert P N, Gotto A M & Fredrickson D S. Familial lipoprotein deficiency. In J H Stanbury, J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease*. McGraw Hill, New York, 1978, p. 544.
- Glueck C J, Gartside P, Fallat R W, Sielski J & Steiner P M. Longevity syndromes: Familial hypobeta and familial hyperalpha lipoproteinemia. *J Lab Clin Med* 88: 941, 1976.

S IgA CHOLERA TOXIN AND ROTAVIRUS ANTIBODY IN HUMAN COLOSTRUM

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ABSTRACT Simhon A, Yolken R H and Mata L (Instituto de Investigaciones en Salud (INISA) Universidad de Costa Rica San Jose Costa Rica) S-IgA cholera toxin and rotavirus antibody in human colostrum. *Acta Paediatr Scand* 68 161 1979.—S-IgA antibodies against cholera toxin and rotavirus were assayed in 43 colostrum samples by means of an enzyme-linked immunosorbent assay (ELISA). All specimens contained specific S-IgA antibodies against both antigens. Significant antibody titres to the antigens were demonstrated in almost all colostrum samples.

KEY WORDS Anti-cholera toxin, anti rotavirus, colostrum.

The aetiology of non specific infantile diarrhoea has been further elucidated by recognition of the pathogenic role of enterotoxigenic *Escherichia coli* (5, 12) and rotavirus (1, 4). The risk of exposure of infants to these agents particularly in developing countries has stimulated study of the protective effect of breast feeding against their infection and associated diarrhoea.

S IgA antibodies to enterotoxins of *Enterobacteriaceae* and *Vibrio cholerae* have been described in human milk by neutralisation assays and the enzyme linked immunosorbent assay (ELISA) (7, 15). Also colostrum of women from Costa Rica, Guatemala and the United States were found to contain considerable amounts of anti rotavirus antibody (21). This report describes a semi quantitative assay of anti-cholera toxin S IgA antibody by ELISA as an alternative to neutralisation techniques. Furthermore additional evidence is presented to confirm that colostrum from Costa Rican women contain significant levels of anti rotavirus antibody.

MATERIALS AND METHODS

Specimens. Colostrum from 43 women aged 16 to 30 years from San José, Costa Rica were obtained one to

72 hours post partum. Mothers were of a medium to low socio-economic status (approximate per capita yearly income US \$70-140). All were at least minimally literate. S-IgA was assayed by the ELISA (17) in a modification developed by Yolken *et al* for rotavirus antigen (18) for labile toxin of *E. coli* (19) and for rotavirus antibody assay (21).

ELISA for cholera toxin antibody. 2.5 ng of cholera toxin (Schwarz Mann Inc Orangeburg N.Y. USA) were added to each well of polyvinyl microtitre plates (Cooke Engineering Co Alexandria Virginia USA) precoated with burro anti-cholera toxin antibody. Six four fold dilutions of each colostrum sample were added in duplicate wells. A conjugate of rabbit anti secretory component (SC) (Dakopatts AS Copenhagen Denmark) and alkaline phosphatase (Sigma Chemical Co St Louis Missouri USA) was then added in each well. Addition of p-nitrophenyl phosphate substrate produced a yellow colour. The reaction was stopped with 3N NaOH and the colour was read at 400 nm in a Beckman DU 7 Spectrophotometer.

ELISA for rotavirus antibody. The procedure described by Yolken *et al* (1) allowed reaction of a guinea pig anti rotavirus serum with a rotavirus positive stool filtrate. The antigen was obtained by experimental infection of a gnotobiotic calf with human rotavirus. This technique was modified as follows: a 10% suspension of pooled stools from Costa Rican children positive for rotavirus by both electron microscopy and ELISA was added to each well of microtitre plates precoated with goat anti human rotavirus antibody (20). Serial four fold dilutions of colostrum were added in duplicate wells and the ELISA was continued as described above.

Controls of ELISA. All colostrum were assayed for the presence of cholera toxin and rotavirus antigen. The anti SC globulin was tested for the presence of antibodies

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MATERIALS AND METHODS

Specimens. Colostrum from 43 women aged 16 to 30 years from San José Costa Rica were obtained one to

72 hours post partum. Mothers were of a medium to low socio-economic status (approximate per capita yearly income US \$70-150). All were at least minimally literate. S IgA was assayed by the ELISA (7, 17) in a modification developed by Yolken et al for rotavirus antigen (20) for faecal toxin of *E. coli* (19) and for rotavirus antibody assay (21).

ELISA for cholera toxin antibody. A 5 µl of cholera toxin (Schwarz Mann Inc Orangeburg N.Y. USA) were added to each well of polyvinyl microtitre plates (Cooke Engineering Co Alexandria Virginia USA) precoated with burro anti-cholera toxin antibody. Six four fold dilutions of each colostrum sample were added in duplicate wells. A conjugate of rabbit anti secretory component (SC) (Dakopatts AS Copenhagen Denmark) and alkaline phosphatase (Sigma Chemical Co St Louis Missouri USA) was then added to each well. Addition of p-nitrophenyl phosphate substrate produced a yellow colour; the reaction was stopped with 3N NaOH and the colour was read at 400 nm in a Beckman DU 2 Spectrophotometer.

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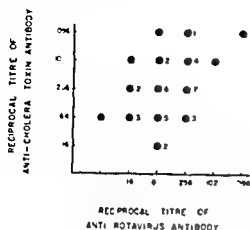


Fig. 1. Scatter diagram of reciprocal anti-cholera toxin and anti-rotavirus antibody titres. 43 colostrum from Costa Rican women 1978. Numerical indicates number of colostrum.

to cholera toxin and rotavirus by means of blocking tests (40). In order to remove spurious antibodies to the various reagents in the tests (i.e. immune sera colostrum) those reagents were treated as described by Volken et al. (22). Addition of colostrum was omitted in negative controls.

ELISA end point titres. To determine the titre of specific S IgA positive/negative (P/N) values were determined for each colostrum by dividing the spectrophotometric absorbance of the diluted sample by that of the negative control. A P/N value of 2.0 or greater was considered confirmatory of the presence of S IgA. Reciprocal antibody titres were defined as the highest dilution with a P/N value of 2.0 or greater.

RESULTS

None of the colostrum samples contained either cholera toxin or rotavirus antigen. The anti-SC globulin failed to block otherwise positive reactions for cholera toxin and rotavirus antigen.

All colostrum samples were shown to contain S IgA antibodies to both cholera toxin and rotavirus antigen (Table 1). The geometric mean titres of anti-cholera toxin and anti-rotavirus antibodies were 264.4 and 114.3 respectively. A scatter diagram of reciprocal titres for each colostrum is presented in Fig. 1. A positive correlating trend was observed.

DISCUSSION

The present findings reveal a high frequency of colostrum specimens with significant titres

of specific S IgA antibodies against cholera toxin. Since there has been no evidence of cholera in Costa Rica in this century it can be advanced that the labile toxin of *E. coli* (or of another *Enterobacteriaceae*) has been the immunogen eliciting anti-cholera toxin antibody. The fact strengthens the resemblance and identity of these toxins (6-13). Labile toxin (LT) of *E. coli* was demonstrated by passive immune hemolysis (3) in 9% of hospitalized diarrhoeic and 6% of non diarrhoeic Costa Rican children over a two year prospective observation (11). Thus a high exposure to LT is observed in Costa Rica which presumably is reflected in high antibody titres. Enterotoxin neutralising activity was demonstrated in a large number of specimens of Guatemalan colostrum and Pakistani mature milk (10). Stohar et al. (15) and Holmgren et al. (7) respectively. Inasmuch neutralisation titre were not determined for the Costa Rican colostrum no comparison can be made with such findings.

The ELISA geometric mean titre of cholera toxin S IgA in Pakistani milk was significantly lower than that of Costa Rican colostrum presumably reflecting differences in concentration between milk and colostrum. The possibility that differences resulted from varying experimental conditions should not be overlooked as crude or partially purified

Table 1. S IgA antibody to cholera toxin and rotavirus

Reciprocal antibody titre	S IgA anti cholera toxin	S IgA anti rotavirus
4	0	1 (2) ^a
16	2 (5) ^a	11 (14)
64	12 (28)	16 (37)
256	15 (35)	15 (35)
1024	11 (26)	4 (9)
4096	3 (7)	1 (2)
Total	43	43

Highest dilution with a P/N value of 2 or greater (determined by dividing the spectrophotometric absorbance of the sample by that of the negative control).

^a Number of colostrum (percentage).

E. coli heat labile enterotoxin was unavailable in our laboratory so commercially purified cholera toxin was utilized in this study. antibody end point titres may have been defined differently in other studies also colostrum from women of different socio economic condition may yield different results. The present findings and those of Yolken et al (21) reveal significant amounts of anti rotavirus antibody in the majority of colostrum specimens regardless of geographical location. Furthermore they showed that rotavirus antibody persisted to detectable levels throughout 24 months of lactation. Studies are under way to measure activity in terms of units of neutralisation per mg of S IgA.

The correlation trend between titres of anti cholera toxin and anti rotavirus antibodies suggests a similar exposure or response in individual mothers to both antigens.

In a model system of human rotavirus infection in lambs (14) passive immunity was studied by oral administration of human anti rotavirus IgG. clinical manifestations were prevented presumably by neutralisation of either the initial inoculum or virus subsequently released by cells. Both Nosocomial outbreaks of rotavirus have been controlled by oral administration of colostrum (16). Similarly exclusively breast fed Indian neonates and young infants of a Guatemalan Mayan village exhibited a lower incidence of gastrointestinal disorders despite frequent exposure to enteropathogens (9, 10).

Regarding anti cholera factors protection against experimental cholera diarrhoea was shown to result from the synergistic action of anti bacterial and anti toxin antibodies (8). More research is needed to further assess the relative protective role of anti bacterial and anti toxin antibodies. Colostrum IgG and IgM antibodies already demonstrated in colostrum from Central American women (18) also may provide added protection against enterotoxigenic and rotavirus infections.

Evidently the study of immune principles active against newly recognised agents and

other virulence factors is of public health significance especially in transitional societies now experimenting a decline in breast feeding. Moreover the adequacy of passive immunity against toxigenic bacteria and rotaviruses must be evaluated in mature milk and in the various phases of lactation.

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REFERENCES

1. Bishop R F, Davidson G P, Holmes I H & Ruck B J. Virus particles in epithelial cells of duodenal mucosa from children with acute non bacterial gastroenteritis. *Lancet* II 1981 1973.
2. Engvall E & Perlmann P. Enzyme linked immunosorbent assay (ELISA). III. Quantitation of specific antibodies by enzyme labeled anti immunoglobulin in antigen coated tubes. *J Immunol* 109 129 1972.
3. Evans D J & Evans D G. Direct serological assay for the heat labile enterotoxin of *Escherichia coli* using passive immune hemolysis. *Infect Immun* 13 604 1977.
4. Flewett T H, Bryden A S & Davies H A. Virus particles in gastroenteritis. *Lancet* II 1497 1973.
5. Gorbach S L, Banwell J G, Chatterjee B D, Jacobs B & Sack R B. Acute undifferentiated human diarrhea in the tropics. I. Alterations in intestinal microflora. *J Clin Invest* 50 881 1971.
6. Holmgren J, Soderlind O & Wadstrom T. Cross reactivity between heat labile enterotoxins of *Vibrio cholerae* and *Escherichia coli* in neutralization tests on rabbit ileum and skin. *Acta Pathol Microbiol Scand Sect B* 81 757 1973.
7. Holmgren J, Hanson L Å, Carlsson B, Lindblad H & Rahimtoola J. Neutralizing antibodies against *Escherichia coli* and *Vibrio cholerae* enterotoxins in human milk from a developing country. *Scand J Immunol* 5 867 1976.
8. Holmgren J & Svennerholm A. Mechanisms of disease and immunity in cholera: a review. *J Infect Dis* 136 (Suppl.) S105 1977.
9. Mata L J & Urrutia J J. Intestinal colonization of breast fed children in a rural area of low socioeconomic level. *Ann NY Acad Sci* 176 93 1971.
10. Mata L J, Kronmal R A, Garcia B, Butler W, Urrutia J J & Munillo S. Breast feeding weaning and the diarrhoea syndrome in a Guatemalan Indian village. In Ciba Foundation Symposium 4 (new series) *Acute diarrhoea in childhood*. Elsevier Excerpta North Holland Amsterdam 1976 p 311.

- 11 Reyes I, Ramírez J A, Evans D J & Mata L. *Escherichia coli* enterotoxigenica en niños hospitalizados de Costa Rica. *Rev Med Hosp Nac Niños* (Costa Rica) in press
- 12 Sack R B, Gorbach S I, Banwell J G, Jacobs B, Chatterjee B D & Mitra R C. Enterotoxigenic *Escherichia coli* isolated from patients with severe cholera-like disease. *J Infect Dis* 173: 378, 1971
- 13 Smith N W & Sack R B. Immunologic cross reactions of enterotoxins from *Escherichia coli* and *Vibrio cholerae*. *J Infect Dis* 127: 164, 1973
- 14 Snodgrass D R, Madeley C R, Wells P W & Angus K W. Human rotavirus in lambs: infection and passive protection. *Infect Immun* 16: 268, 1977
- 15 Stollar O A, Pelley E P, Karmali M A, Green I, Klaus M H & Carpenter C C J. Secretory IgA against enterotoxins in breast milk. *Lancet* i: 1258, 1976
- 16 Totterdahl B M, Chrystie I I & Banatvala J F. Rotavirus infection in a maternity unit. *Arch Dis Child* 51: 924, 1976
- 17 Voller A, Bidwell D & Birtlett A. Microplate enzyme immunoassays for the immunodiagnosis of virus infections. In N. Rose & H. Friedman (eds.) *Manual of clinical immunology*. American Society for Microbiology, Washington D C, 1976, p. 506
- 18 Wyatt R B, García B, Ciceres A & Mata L J. Immunoglobulins and antibodies in colostrum and milk of Guatemalan Mayan women. *Arch Lat Am Nutr* 27: 629, 1972
- 19 Yolken R H, Greenberg H B, Merson M H, Sack R B & Kapikian A Z. Enzyme linked immunosorbent assay for detection of *Escherichia coli* heat labile enterotoxin. *J Clin Microbiol* 6: 439, 1972
- 20 Yolken R H, Kim H W, Clem T, Wyatt R G, Kalica A R, Chanock R M & Kapikian A Z. Enzyme linked immunosorbent assay (ELISA) for detection of human reovirus like agent of infantile gastroenteritis. *Lancet* ii: 763, 1977
- 21 Yolken R H, Mata L, García B, Urrutia J J, Wyatt R G, Chanock R M & Kapikian A Z. Secretory antibody directed against rotavirus in human milk: measurement by means of an enzyme linked immunosorbent assay (ELISA). *J Pediatr* in press
- 22 Yolken R H, Wyatt R G & Kapikian A Z. ELISA for rotavirus. *Lancet* ii: 819, 1977

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DIETARY HABITS AND SERUM LIPIDS DURING FIRST 4 YEARS OF LIFE

A Study of 95 Danish Children

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ABSTRACT Andersen G E, Lifschitz C and Friis Hansen B (Neonatal Department Rigshospitalet Copenhagen Denmark) Dietary habits and serum lipids during first 4 years of life. A study of 95 Danish children. *Acta Paediatr Scand* 68: 165, 1979. — Early infant feeding habits, current dietary intake and serum lipids were investigated in 31 infants, age 6–10 months and 64 children, age 3–4 years. In the infants there was a correlation between serum lipid levels and the amount of saturated fat and the P/S ratio of the diet. No such correlation was found in the 3–4 year old children. Neither was there any correlation between the type and duration of early infant feeding and subsequent serum lipid levels. In both the infants and the 3–4 year old children serum cholesterol concentration correlated with the serum cholesterol concentration in each of the parents.

KEY WORDS Cholesterol, triglyceride, lipoprotein, nutrition, childhood.

Hypercholesterolemia is a well documented risk factor in the development of coronary heart disease (1–3). In population studies serum cholesterol concentration has been found to correlate with the proportion of dietary calories derived from fat (4–9), particularly saturated fats (10). However, studies which have tried to relate individual nutrient intake to serum cholesterol levels have failed to demonstrate any such correlation (11–15). The pioneer work of Brown & Goldstein (16) has shown that a substrate concentration of about 0.062 mmol/l of LDL C is sufficient to satisfy extrahepatic parenchymal cellular cholesterol requirements for membrane and hormone synthesis via high affinity LDL receptors. The ratio of lymph to serum concentrations of LDL C has been calculated to be in the order of 1:10 (17). Therefore a lymph LDL C concentration of 0.062 mmol/l corresponds to a serum LDL C level of about 0.62 mmol/l, which is close to the median level of cord serum LDL C found in normal newborns (18) and in eight mammalian species which are not naturally susceptible to atherosclerosis (19). In Western adults, however, the mean serum

LDL C concentration is about 5 times higher i.e. 3.23 mmol/l (20). The question so far unresolved is how and when this five fold increase from birth to adult life takes place. In order to contribute to the elucidation of this problem we here present data from a study of 95 normal children, 64 of whom have been followed from birth to age 3–4.

MATERIAL

31 infants (13 girls and 18 boys) aged 6–10 months were randomly selected from our screening program for hyperlipoproteinemia (21) and 69 children (31 girls and 38 boys) aged 3–4 years were taken from a sample of 303 normal full term newborns who have earlier been described in detail (22). None of the 100 children here investigated have familial hypercholesterolemia (FH). The study and its purpose was explained to the parents and their consent obtained. In cases of intercurrent disease the investigation of the child was postponed for a minimum of 2 weeks after the disappearance of symptoms to ensure that any possible drop in appetite and weight had returned to normal. The daily nutritional intake of each child was evaluated by

Abbreviations T-C=total cholesterol, VLDL-C=very low density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, TG=triglyceride, P/S=ratio between the amount of polyunsaturated and saturated fat in the diet.

Table 1 Distribution of data in 6-10 month old infants ($n=31$)

Percentile values	10	50	90
Age (months)	6.2	8.0	10.0
Weight (kg)	7.1	8.7	10.0
Weeks of breast feeding	0	6	18
Weeks of formula feeding	0	8	17
Weeks of diluted cow's milk feeding	0	4	8
<i>Current daily dietary intake</i>			
Kcal			
Protein (g)	704	918	1265
Carbohydrates (g)	290	430	630
Total fat (g)	840	1110	2260
Saturated fat (g) (S)	152	300	536
Polyunsaturated fat (g) (P)	62	162	250
P/S	13	3.0	5.8
Cholesterol (mg)	0.09	0.20	0.40
	82	170	267
<i>Current serum lipids (mmol/l)</i>			
T-C	3.36	4.22	5.52
VLDL C	0.06	0.20	0.47
LDL C	1.72	2.45	3.49
HDL C	1.14	1.67	2.19
TG	0.57	0.87	1.40

a one week dietary protocol written by the mother in combination with a final 24 hour dietary recall interview made by a dietician. The amounts of daily nutrients were then calculated according to a coded list of Danish food items (23). Furthermore the mother and the dietician examined the child's health chart in which details of the type and duration of early infant feeding had been recorded by the visiting health nurse in collaboration with the mother. On the morning of the interview a sample of venous blood was drawn from each child after an 8 hour fast (infants) or a 10-12 hour fast (3-4 year old children). In the parents a venous blood sample had been drawn at an earlier date after a 12 hour fast. The serum was separated and stored at 4°C and the analyses begun within 24 hours. In 2 children no venous blood sample could be taken and in 3 children information on dietary intake was incomplete thus resulting in a final total of 95 children.

METHODS

Serum T-C was measured manually in duplicate using the enzymatic method described by Roschlau et al (24). Four Preciset cholesterol standards were included in over 100 separate runs. The coefficient of variation was 4.4% (for the 0.32 mmol/l cholesterol Preciset) 1.7% (for the 2.59 mmol/l cholesterol Preciset) 1.9% (for the 3.88 mmol/l cholesterol Preciset) and 2.1% (for the 11.35 mmol/l cholesterol Preciset). Serum TG was measured manually in duplicate using the enzymatic method described by Eggstein & Kreutz (25). Two Liponorm triglyceride standards were included in over 100 separate runs. The coefficient of variation was 3.6% (for the 0.4 mmol/l triglyceride Liponorm) and 2.0% (for the 2.50 mmol/l triglyceride Liponorm). VLDL LDL C was meas-

ured manually after CaCl_2 heparin precipitation in cate as described by Andersen & Gry Nielsen. VLDL C was measured manually in duplicate after centrifugation in a 40.3 rotor Beckman type L ultratuge at 10°C at 40000 r.p.m. for 20 hours. Tube 1 was used. HDL values were calculated as the difference between (T-C)-(VLDL+LDL C).

Statistics

The distribution of data in the two groups of children determined by calculating the percentile values. The relationship between the child's current serum lipid level, his past serum cholesterol, serum cholesterol in parents, early infant feeding and daily nutrient intake evaluated by calculating the Spearman rank correlation coefficients and conducting the appropriate T-test described by Siegel (27). The Mann-Whitney test was used for comparing serum lipid values in the 6-10 month infants and the 3-4 year old children as described by Siegel (27).

RESULTS

6-10 months old infants

In Table 1 the distributions of age, weight, sex and duration of early infant feeding, current dietary intake and serum lipids are given for the 31 infants. Only 2 infants were purely breast fed for >20 weeks (28 and 32 weeks respectively). They were still being breast fed at the time their serum lipids were determined. The

Table 2 6-10 month old infants Correlations between current serum TC and serum TC at birth serum TC of the parents early infant feeding and current dietary intake ($n=31$)

	<i>r</i>	<i>P</i>
Child's own cord serum TC	-0.080	NS
Child's own cord serum LDL-C	0.771	NS
Mother's age-corrected TC	0.335	<0.05
Father's age-corrected TC	0.311	<0.05
Weeks of breast feeding	0.006	NS
Weeks of formula feeding	0.079	NS
Weeks of diluted cow's milk feeding	-0.049	NS
<i>Current daily dietary intake</i>		
Kcal	-0.141	NS
Protein	-0.786	NS
Carbohydrates	-0.54	NS
Total fat	0.85	NS
Saturated fat	0.372	<0.05
Polyunsaturated fat	-0.770	NS
P/S	-0.630	<0.0001
Cholesterol	0.707	NS

infants were purely formula fed for 20-24 weeks. The remaining 26 infants were first breast fed and then received formula and/or diluted cow's milk. In Table 2 the relationships between the current serum TC of the child and his cord serum TC and LDL-C the age

adjusted serum TC of his parents the duration of breast formula and diluted cow's milk feeding and the amount of nutrients in his current diet are presented. A correlation is seen to exist between the current serum TC of the child and that of his parents. Furthermore the current serum TC level is seen to correlate directly with the amount of saturated fat and inversely with the P/S ratio of the diet. Exactly the same pattern emerged when the infant's current serum LDL-C was compared to the factors given in Table 2.

As for serum TG an inverse correlation was found with the amount of fat in the diet ($r=-0.436$ $p<0.01$) and the amount of dietary cholesterol ($r=-0.479$ $p<0.005$) but not with the amount of dietary carbohydrate ($r=0.104$ $p>0.05$).

No difference was found in the distribution of any of the serum lipids in the 13 girls and 18 boys.

3-4 year old children

In Table 3 the distribution of age weight type and duration of early infant feeding current dietary intake and serum lipids are given for

Table 3 Distribution of data in 3-4 year old children ($n=64$)

Percentile values	5	10	50	90	95
Age (months)	36	37	47.5	50	50
Weight (kg)	14.5	15.1	17.5	17.7	21.4
Weeks of breast feeding	0	0	8	16	20
Weeks of formula feeding	0	0	8	10	10
Weeks of diluted cow's milk feeding	0	0	0	17	14
<i>Current daily dietary intake</i>					
Kcal	981	1061	1500	1600	1811
Protein (g)	78	46	65	87	90
Carbohydrates (g)	96	103	157	236	276
Total fat (g)	36	40.0	66.0	97.4	125.2
Saturated fat (g)	14.6	17.7	25.5	45.0	53.0
Polyunsaturated fat (g)	3.0	4.9	8.9	15.1	21.8
P/S	0.15	0.17	0.37	0.58	0.68
Cholesterol (mg)	134	110	90	449	502
<i>Current serum lipids (mmol/l)</i>					
TC	3.40	3.69	4.77	5.75	6.07
LDL-C	0.04	0.05	0.12	0.28	0.34
LDL-C	1.51	1.97	4.7	3.43	3.87
HDL-C	1.6	1.49	2.01	2.57	2.87
TG	0.49	0.51	0.65	0.89	1.12

Table 4 3-4 year old children Correlations between current serum T C and serum T C at birth 7-9 and 14-19 months serum T C of the parents early infant feeding and current dietary intake (n=64)

	r	P
Child's cord serum T C	-0.144	N S
Child's serum T C at 7-9 months	0.450	<0.0005
Child's serum T C at 14-19 months	0.517	<0.00005
Mother's age corrected T C	0.380	<0.001
Father's age corrected T C	0.350	<0.005
Weeks of breast feeding	-0.091	N S
Weeks of formula feeding	0.057	N S
Weeks of diluted cow's milk feeding	0.038	N S
<i>Current daily dietary intake</i>		
Carbs	0.021	N S
Protein	0.004	N S
Carbohydrates	0.076	N S
Total fat	-0.066	N S
Saturated fat	0.057	N S
Polyunsaturated fat	-0.005	N S
P/S	-0.150	N S
Cholesterol	0.067	N S

the 64 children. In Table 4 the relationships between the current serum T C of the child and his past serum T C, the age corrected serum T C in each of his parents, the duration of breast, formula and diluted cow's milk feeding and the amount of daily nutrients of his diet are presented. Correlations are seen to exist between the current serum T C level of the child and his serum T C at age 7-9 and 14-19 months and the serum T C of each of his parents. However, no correlations were found between either his current serum T C and the duration of breast, milk, formula and diluted cow's milk feeding or between his current serum T C and the individual nutrients of his diet. Exactly the same pattern emerged when the child's current serum LDL C was compared to the factors given in Table 4.

As for serum TG a correlation was found with the serum TG at age 7-9 months ($r=0.282$, $p<0.05$) and at age 14-19 months ($r=0.291$, $p<0.05$). No correlation was found between serum TG and the individual nutrients of the diet. No difference was found in the distribu-

tion of any of the serum lipids in the 28 girls and 36 boys.

A comparison of the serum lipids in the two groups of children showed that in the 3-4 year old children serum T C was higher ($p<0.05$), TG lower ($p<0.001$), VLDL-C lower ($p<0.005$) and HDL C higher ($p<0.001$) than in the 6-10 months old infants. No difference was found between serum LDL-C levels in the two groups of children ($p=0.836$).

DISCUSSION

The median value for serum T C (4.22 mmol/l) in our 6-10 months old infants is of the same order of magnitude as the mean values for serum T-C (3.78-5.06 mmol/l) in infants on various diets found by Fomon & Bartels (28), Paupe et al (29), Woodruff et al (30), Darmady et al (31) and Fosbrooke & Wharton (32).

The median value for serum T C (4.77 mmol/l) in our 3-4 year old children is 16% higher than the mean value for serum T-C (3.98 mmol/l) in 3 year old children reported by Friedman & Goldberg (33). This discrepancy may partly be due to the use of different methods for measuring T C. Our finding that there is no association between the type and duration of early infant feeding and subsequent serum T C, be it after as short as 3 months or as long as 3 years, confirms the results of Friedman & Goldberg (34) and Glueck et al (35) and is one further proof that the hypothesis of Reiser et al (36) is incorrect. Their hypothesis, derived from experiments with infant male rats and pigs, was that the feeding of a high cholesterol diet to the human infant might cause subsequent low values of serum T C, based on the speculation that in early high cholesterol diet might induce life long high enzyme activities in cholesterol catabolism.

Our finding that in 6-10 months old infants there is a direct association between the amount of saturated fat and an inverse relationship between the P/S ratio of the diet and the level of serum T C is in agreement with what has been reported by Fomon & Bartels

28) Goalwin & Pomeranze (37) Paupe et al
29) Lowe et al (38) Glueck et al (35) Dar
mady et al (31) and Friedman & Goldberg
(34)

Our finding that beyond infancy there is no association between the amounts of daily nutrients and the concentration of serum T C confirms the results of Hard & Esselbaugh (39) and Hitchcock & Gracey (15) and Weidman et al (40) in older children

This report is the first which has shown that the increase in serum T C levels between infancy (6-10 months) and age 3-4 years is caused by an increase in HDL C Serum LDL C was found to be the same in the two age groups

In conclusion we have found that about 3/5 of the 5 fold increase in serum LDL C from birth to adult life takes place in early infancy. In infants the type and amount of fat in the diet seem to influence the level of serum T C and TG. This influence however seems to be lost beyond infancy since in 3-4 year old children serum T C and TG do not correlate with dietary intake. Both during infancy and later childhood there is a correlation between serum T C of the child and his parents. This might be interpreted in the following way: During infancy serum T C seems to be under at least both genetic and dietary influence. Beyond infancy however the dietary influence subsides whereas the genetic influence seems to persist.

ACKNOWLEDGEMENT

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REFERENCES

- Kannel W B, Castelli W P, Gordon T & Marmara M. Serum cholesterol lipoproteins and the risk of coronary heart disease. The Framingham study. *Ann Int Med* 74: 1 1971
- Chapman J M & Mavrey F J. The interrelationship of serum cholesterol, hypertension, body weight and risk of coronary disease. *J Chron Dis* 17: 933 1964
- Rosenman R H, Friedman M, Strauss M, Jenkins C H, Zyzanski S J & Wurm M. Coronary heart disease in the Western Collaborative Group Study. *J Chron Dis* 23: 173 1970
- Keys A, Anderson J T & Grande F. Prediction of serum cholesterol response of man to changes in fats in the diet. *Lancet* II: 959 1957
- Connor W E, Stone H H & Hodges R E. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. *J Clin Invest* 43: 1691 1964
- National diet-heart study research group. The national diet-heart study final report. *Circulation* 37 (Suppl 1): 1 1968
- Keys A. Coronary heart disease in seven countries. *Circulation* 41 (Suppl 1): 1 1970
- Kato H, Tilloison J, Nichaman M Z, Rhoads G & Hamilton H. Epidemiological studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. *Am J Epidemiol* 97: 372 1973
- Hankin J, Reed D, Labarthe D, Nichaman M & Stallones R. Dietary and disease patterns among Micronesians. *Am J Clin Nutr* 23: 346 1970
- Atherosclerosis study group. Primary prevention of the atherosclerotic diseases. Report of intersociety commission for heart disease resources. *Circulation* 42 (Suppl A): 1 1970
- Kannel W & Gordon T. The Framingham study. An epidemiological investigation of cardiovascular diseases. Section 24. The Framingham diet study. Diet and the regulation of serum cholesterol. U.S. Government Printing Office 1970
- Kahn H, Medahe J, Neufeld H, Riis E, Balogh M & Groen J. Serum cholesterol. Its distribution and association with dietary and other variables in a survey of 10000 men. *Israel J Med Sci* 5: 1117 1969
- Stulb S, McDonough T, Greenberg H & Hames C G. The relationship of nutrient intake and exercise to serum cholesterol levels in white males in Evans County, Georgia. *Am J Clin Nutr* 16: 238 1965
- Nichols A B, Ravenscroft C, Langheir H E & Ostrander L H. Daily nutritional intake and serum lipid levels. The Tecumseh study. *Am J Clin Nutr* 29: 1384 1976
- Hitchcock N E & Gracey M. Diet and serum cholesterol. An Australian family study. *Arch Dis Child* 52: 790 1977
- Goldstein J L & Brown M. Atherosclerosis. The low-density lipoprotein receptor hypothesis. *Metabolism* 26: 1257 1977
- Reichl D, Simons L A & Myant N B. The lipids and lipoproteins of human peripheral lymph: with observations on the transport of cholesterol from plasma and tissues into lymph. *Clin Sci Mol Med* 45: 313 1973
- Andersen G E & Frus Hansen H. Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone treated newborns of varying gestational age. *Acta Paediatr Scand* 66: 355 1977

Table 4 3-4 year old children Correlations between current serum TC and serum TC at birth 7-9 and 14-19 months serum TC of the parents early infant feeding and current dietary intake (n=64)

	r	P
Child's cord serum TC	-0.144	NS
Child's serum TC at 7-9 months	0.450	<0.0005
Child's serum TC at 14-19 months	0.517	<0.00005
Mother's age corrected TC	0.340	<0.001
Father's age corrected TC	0.340	<0.005
Weeks of breast feeding	-0.091	NS
Weeks of formula feeding	0.057	NS
Weeks of diluted cow's milk feeding	0.034	NS
Current daily dietary intake		
Kerls	0.021	NS
Protein	0.004	NS
Carbohydrates	0.076	NS
Total fat	-0.066	NS
Saturated fat	0.057	NS
Polyunsaturated fat	-0.005	NS
P/S	-0.150	NS
Cholesterol	0.067	NS

the 64 children. In Table 4 the relationships between the current serum TC of the child and his past serum TC, the age corrected serum TC in each of his parents, the duration of breast, formula and diluted cow's milk feeding and the amount of daily nutrients of his diet are presented. Correlations are seen to exist between the current serum TC level of the child and his serum TC at age 7-9 and 14-19 months and the serum TC of each of his parents. However, no correlations were found between either his current serum TC and the duration of breast milk, formula and diluted cow's milk feeding or between his current serum TC and the individual nutrients of his diet. Exactly the same pattern emerged when the child's current serum LDL C was compared to the factors given in Table 4.

As for serum TG a correlation was found with the serum TG at age 7-9 months ($r=0.282$, $p<0.05$) and at age 14-19 months ($r=0.291$, $p<0.05$). No correlation was found between serum TG and the individual nutrients of the diet. No difference was found in the distribu-

tion of any of the serum lipids in the 28 girls and 36 boys.

A comparison of the serum lipids in the two groups of children showed that in the 3 year old children serum TC was high ($p<0.05$), TG lower ($p<0.001$), VLDL lower ($p<0.005$) and HDL C higher ($p<0.05$) than in the 6-10 months old infants. No difference was found between serum LDL levels in the two groups of children ($p=0.83$).

DISCUSSION

The median value for serum TC (4.22 mmol/l) in our 6-10 months old infants is of the same order of magnitude as the mean values for serum TC (3.78-5.06 mmol/l) in infants on various diets found by Fomon & Bartels (19), Prupe et al (29), Woodruff et al (30), Darby et al (31) and Fosbrooke & Wharton (32).

The median value for serum TC (4.22 mmol/l) in our 3-4 year old children is 10% higher than the mean value for serum TC (3.98 mmol/l) in 3 year old children reported by Friedman & Goldberg (33). This discrepancy may partly be due to the use of different methods for measuring TC. Our finding that there is no association between the type and duration of early infant feeding and subsequent serum TC, be it after as short as 3 months or as long as 3 years, confirms the results of Friedman & Goldberg (34) and Glueck et al (35). It is one further proof that the hypothesis of Serfaty et al (36) is incorrect. Their hypothesis, derived from experiments with infant male rats and pigs, was that the feeding of a high cholesterol diet to the human infant might cause subsequent low values of serum TC based on the speculation that an early high cholesterol diet might induce life long high enzyme activities in cholesterol catabolism.

Our finding that in 6-10 months old infants there is a direct association between the amount of saturated fat and an inverse relationship between the P/S ratio of the diet and the level of serum TC is in agreement with what has been reported by Fomon & Bar-

H₂ BREATH TESTS DURING DIARRHEA

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ABSTRACT Solomons N W García R Schneider R Viteri F and Argueta von Kaenel V (Division of Human Nutrition and Biology Institute of Nutrition of Central America and Panama and Department of Pediatrics San Juan de Dios General Hospital Guatemala City Guatemala) H₂ breath test during diarrhea *Acta Paediatr Scand* 68 171 1979.—The peak rise in breath hydrogen and the volume of excess pulmonary excretion of hydrogen in response to a 10 g dose of the non absorbable disaccharide lactulose was significantly lower in children with active gastroenteritis and diarrhea than in non diarrheal controls. Thus despite the fact that the H₂ breath test is a convenient non invasive technology for use in children it cannot be recommended for measuring carbohydrate malabsorption in individuals with active on-going episodes of diarrhea.

KEY WORDS Hydrogen breath test carbohydrate malabsorption infantile diarrhea lactulose

When ingested carbohydrate is exposed to certain colonic bacteria fermentation results in the intraintestinal production of hydrogen (H₂). Some of this H₂ is excreted by the lungs. Since a linear and relatively constant relationship between the amount of non absorbed carbohydrate and the rate of H₂ excretion has been demonstrated (1) clinical carbohydrate malabsorption tests based on breath analysis have been developed. The innocuous non invasive nature of the collection procedure as compared with other techniques for quantifying carbohydrate malabsorption has encouraged application of the H₂ methodology in young children (2-4, 6).

We have been interested in determining the amount of nutrient loss which can occur as the result of acute infantile gastroenteritis and felt that the H₂ breath test would be useful in the evaluation of this problem. However our initial experience with administering a 15 g oral glucose dose to infants with intractable diarrhea and clinical monosaccharide intolerance or with giving 1.75 g of lactose per kg to children with acute enteritis failed to show the expected rise in breath H₂ where

large amounts of fecal reducing substances could be detected in the stools (Solomons García and Viteri unpublished). Moreover in some institutionalized children followed prospectively a drastic reduction in the usual rate of pulmonary H₂ elimination on a normal diet accompanied episodes of diarrhea (Schneider unpublished). These curious results led us to examine the question of whether or not the presence of diarrhea itself was affecting the pulmonary excretion of H₂.

PATIENTS AND METHODS

Five pre school aged children without diarrhea in the Clinical Research Center of the Division of Human Nutrition and Biology Institute of Nutrition of Central America and Panama served as controls. Ten children aged 6 months to 3 years selected at random from among patients admitted to the Rehydration Unit of the San Juan de Dios General Hospital with acute gastroenteritis had ongoing active diarrhea. Only 3 of the latter subjects had received antibiotics prior to study. All subjects received 10 g of the non absorbable disaccharide lactulose. Samples of expired air were collected before and at 30-min intervals following the administration of the lactulose dose for a total of 4 hours and the breath H₂ concentration was measured by gas chromatography as previously described (5). The pulmonary excretion of

- 19 Mills G I & Taylor C L The distribution and composition of serum lipoproteins in eighteen animals *Comp Biochem Physiol* 40B 489 1971
- 20 Fredrickson D S & Levy R I In J B Stanbury J B Wyngarden J D S Fredrickson (eds) *The metabolic basis of inherited disease* McGraw Hill New York 1972 p 547
- 21 Andersen G E Lous P & Fris Hansen B screening for hyperlipoproteinemia in 10000 Danish newborns *Acta Paediatr Scand* 68 1979 In press
- 22 Andersen G E & Fris Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics* 57 214 1976
- 23 Helms P *Fødevaretabeller* Akademisk Forlag Copenhagen 1975
- 24 Röschliu P Berni F & Gruber W Enzymatische Bestimmung des Gesamt Cholesterins im Serum *Z Klin Chem Klin Biochem* 17 4 1974
- 25 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe *Klin Wochenschr* 44 262 1966
- 26 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia: Methods for direct estimation of cord serum VLDL LDL *Clin Chim Acta* 66 29 1976
- 27 Siegel S In S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 p 203 and 116
- 28 Fomon S J & Bartels D J Concentrations of cholesterol in serum of infants in relation to diet *Am J Dis Child* 99 27 1960
- 29 Piuppe J Colin J & Hishorson Politis E La cholestérolémie du nouveau né et du nourisson normaux jusqu'à 2 ans *Arch Fr Pédiatr* 70 513 1963
- 30 Woodruff C W Bailey M C Davis J T Rogers N & Coniglio J G Serum lipids in breast fed infants and in infants fed evaporated milk *Am J Clin Nutr* 14 83 1964
- 31 Darmady J M Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during first year of life *Br Med J* 11 685 1972
- 32 Fosbrooke A S & Wharton H A Added lactose and added sucrose cow's milk formulae in relation to low birthweight babies *Arch Dis Child* 50 4 1975
- 33 Friedman G & Goldberg S J An evaluation of safety of a low saturated fat low-cholesterol diet given in infancy *Pediatr* 48 655 1976
- 34 Friedman G & Goldberg S J Concurrent and subsequent serum cholesterol of breast and formula infants *Am J Clin Nutr Invest* 18 47 1974
- 35 Glueck C J Tsang H Balistren M & Fa'fat Plasma and dietary cholesterol in infancy: Effect of early low or moderate dietary cholesterol intake on subsequent response to increased dietary cholesterol *Metabolism* 21 1181 1972
- 36 Reiser R & Sidelman Z Control of serum cholesterol homeostasis by cholesterol in the milk of suckling rat *J Nutr* 107 1009 1972
- 37 Goalwin A & Pomeranze J Serum cholesterol studies in infants *Arch Paediatr* 79 48 1964
- 38 Lowe C U Mosovich L L & Pessin V Effect of protein level and type of heat treatment of formulas on growth and maturation of infants *Pediatr* 64 666 1964
- 39 Hard M M & Esselbaugh N C Nutritional status of adolescent children IV Cholesterol relations *Am J Clin Nutr* 8 346 1960
- 40 Weidman W H Elveback L R Nelson R Hodgson P A & Ellefson R D Nutrient intake and serum cholesterol level in normal children years of age *Pediatrics* 61 754 1974

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RENAL FUNCTIONAL CHANGES IN ACUTE GLOMERULONEPHRITIS IN CHILDREN A ONE YEAR FOLLOW UP

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ABSTRACT Aperia A Bergstrand A Broberger O Linne T and Wasserman J (Department of Paediatrics Karolinska Institutet St Goran's Children's Hospital Department of Pathology Karolinska Institutet Huddinge Hospital and the Central Microbiological Laboratory Stockholm County Council Stockholm Sweden) Renal functional changes in acute glomerulonephritis in children A one-year follow up *Acta Paediatr Scand* 68 173 1979.—Renal function was studied in three patients with post streptococcal four patients with IgA and one patient with non streptococcal proliferative glomerulonephritis (GN) at the onset of the disease and two six and 12 months later Renal biopsies were performed at the onset of the disease and 12 months later Standard clearance techniques were used for the functional studies The latter were performed during hydropenia and continuous isotonic saline infusion During hydropenia the GFR was uniformly depressed shortly after the onset of the disease but it normalized during the following two months The filtration fraction was depressed in poststreptococcal GN at the onset and it normalized with the GFR In IgA GN the filtration fraction remained within normal limits during the entire course of the illness The natriuretic response to isotonic saline volume expansion was low in all patients at the onset of the disease but normalized in post-streptococcal and IgA GN during the one year follow up In spite of normalized renal function biopsy findings in IgA GN were unchanged 12 months later An episode of macroscopic hematuria in one patient with IgA GN at the six month investigation had no apparent effect on renal function

KEY WORDS Post-streptococcal glomerulonephritis IgA glomerulonephritis renal function Isotonic saline infusion one-year clinical course

Glomerulonephritis (GN) is the most common cause of chronic renal failure in children (14) and adults (6) The natural course of the different types of GN is incompletely known however and our ability to identify patients at risk of developing progressive renal damage is unsatisfactory The pathophysiology of the hypertension seen in acute and chronic GN also needs to be clarified Systematic prospective studies of patients with well-defined GN from the onset of the disease should help us to better understand the disease and its consequences and enable us to handle clinical problems more satisfactorily In order to see what information could be obtained by an intensive one year follow up study of acute GN eight children were serially examined from

the onset of the disease and at two six and 12 months Data were obtained on the clinical course renal function and renal pathology

MATERIAL

The studies were performed during a two-year period (1973-75) on patients who were admitted to St Goran's Children's Hospital with the preliminary diagnosis of acute GN The patients were admitted to the study if they fulfilled the following criteria 1) no previous history of renal disease 2) no signs or symptoms of systemic disease such as anaphylactoid purpura or systemic lupus erythematosus 3) acute onset of longstanding macroscopic hematuria with proteinuria for at least one week and 4) negative urine culture and normal intravenous pyelogram The diagnosis of acute glomerular disease was further confirmed by the clinical course laboratory findings and the results of renal biopsy

Eight patients aged 4.5 to 9.5 years were studied Three

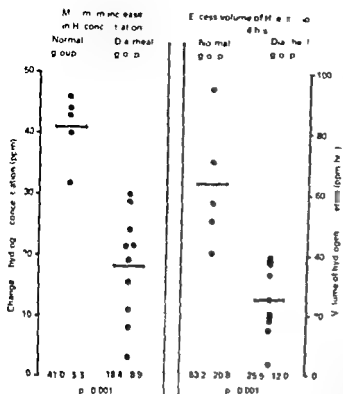


Fig. 1 Scattergram illustrating the breath hydrogen response to ingestion of 10 g of lactulose in healthy children and in children with diarrhea. Hydrogen excretion has been quantified both as the maximum increase in H_2 concentration in ppm and as the excess volume of H_2 in ppm hrs over 4 hours.

H_2 was compared in two ways: 1) as the maximum increase in H_2 concentration in ppm and 2) as the volume of H_2 eliminated during the 4-hour period in ppm hrs.

RESULTS AND DISCUSSION

As shown in Fig. 1, a significant reduction ($p < 0.001$) in H_2 excretion to the same 10 g dose of lactulose was seen among children with acute diarrhea as compared with normal control children. The explanation is clear but several mechanisms could be involved. First, quantitative or qualitative changes in fecal flora during diarrhea could reduce the critical mass of appropriate bacteria necessary for fermentation of the carbohydrate substrate. Alternatively, changes in colonic motility during active diarrhea could change the partition of colonic H_2 excretion between breath and flatus with a proportionately greater amount of intestinal H_2 being excreted by rectum. We and others previously shown that oral antibiotics can interfere with the bacterial metabolism and H_2 excretion from carbo-

hydrates (3, 5) but in only 3 of the 10 subjects was prior use of antibiotics a factor.

The H_2 breath test can be a valuable and reliable technology for the study of carbohydrate malabsorption. Its non-invasive nature makes it particularly suited to studies in young children. The important conclusion of the present study is that standard H_2 breath test criteria for carbohydrate malabsorption based on normal subjects cannot be reliably applied to individuals with active gastroenteritis. This would seem to render the H_2 breath analysis techniques inapplicable to the study of carbohydrate absorption in diarrheal disease.

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REFERENCES

1. Bond J. H. & Levitt M. D. Use of pulmonary hydrogen (H_2) measurements to quantitate carbohydrate absorption. Study of partially gastrectomized patients. *J Clin Invest* 51: 1219, 1972.
2. Maffei H. V., Metz G. L. & Jenkins D. J. A. Hydrogen breath test: Adaptation of 2 simple techniques to infants and children. *Lancet* i: 1110, 1976.
3. Murphy E. L. & Calloway D. H. The effect of antibiotic drugs on the volume and composition of intestinal gas from beans. *Am J Dig Dis* 17: 6, 1972.
4. Perman J. A., Barr R. G. & Watkins J. B. Sucrose malabsorption in children: Non-invasive diagnosis by interval breath hydrogen determination. *J Pediatr* (in press).
5. Solomons N. W., Viten F. E. & Hamilton L. H. Application of a simple gas chromatographic technique for measuring breath hydrogen. *J Lab Clin Med* 9: 846, 1977.
6. Solomons N. W., Viten F. E. & Rosenberg I. H. Development of an interval sampling hydrogen (H_2) breath test for carbohydrate (CHO) malabsorption in children: Evidence for a circadian pattern of H_2 concentration. *Pediatr Res* 12: 816, 1978.

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Table 1 Glomerular filtration rate (ml/1.73 m. BSA/min) in acute glomerulonephritis

Values are mean \pm S.E.M. HP=hydropenia VE=volume expansion

	Onset		1 st months later		Difference onset-12 months later	
	HP	VE	HP	VE	HP	VE
Poststreptococcal GN	71.9 \pm 5.4	92.3 \pm 18.2	99.8 \pm 1.4	100.9 \pm 6.7	+6.9 $p<0.01$	+8.8 NS
IgA GN	74.9 \pm 6.6	107.3 \pm 13.7	96.5 \pm 5.7	103.4 \pm 3.9	+21.6 $p<0.01$	-3.9 NS
Non streptococcal proliferative GN	68.0	91.7	66.4	101.9	-1.6	+10.2

RESULTS

Renal biopsy

Biopsy material was available only from one patient with PSGN (T.E.) one month after the onset of the disease. Slight mesangial hypercellularity and increased amounts of matrix was seen with segmental and focal distribution. In some glomeruli the capillary tuft had increased lobulation. IFL was positive only for C3 with mesangial localization.

Three (P.H., K.J., O.M.) of the patients with IgA GN were biopsied one to three months after the onset of hematuria. In all cases there was a slight to moderate increase

in mesangial matrix without hypercellularity in most glomeruli. After 12 months the lesions were unchanged in two of the patients (P.H., K.J.) and in the third (O.M.) an additional segmental sclerosis was found. The fourth patient (A.N.) who was biopsied for the first time at 12 months had minimal changes. IFL examination showed granular deposits of IgA, IgG and C3 predominantly in the mesangium but also along capillary glomerular basement membranes. In two cases IgM was present (P.H., K.J.). No change in the IFL pattern was noted between the first and second biopsies (P.H., K.J.).

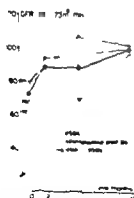


Fig. 1a The GFR during HP in PS and non streptococcal proliferative GN (open circles) shortly after the onset of the disease and two six and 1 months later. The individual values are connected by thin continuous lines and by an interrupted line in non streptococcal proliferative GN. The closed circles and connecting thick line represent the means in PS GN.

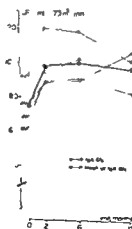


Fig. 1b The GFR during HP in IgA GN shortly after the onset of the disease and two six and 1 months later. The means (closed circles) and individual values (open circles) are shown.

patients (one boy, two girls) were classified as post streptococcal GN, four (three boys, one girl) as IgA GN and one (boy) as non streptococcal proliferative GN. The criteria used for the subel classification were high ASO titers and C3 depression in post streptococcal GN (PSGN), recurrent microscopic hematuria and deposits of IgA predominantly in the glomerular mesangium in IgA GN and stationary ASO titers, long-standing depression of C3 and renal biopsy findings in non streptococcal proliferative GN.

Two (R, F, A, H) of the three patients with post streptococcal GN had increased S_{urea}N during the first days of the disease. This was in one case (R, F) associated with oliguria and edema but not hypertension. All the patients showed initial good recovery. Blood pressure, blood volume, bromide space, recumbent renin and aldosterone excretion were essentially within normal limits during the entire follow up period. Urinalysis was normal in all cases 12 months after the onset of the disease. In connection with a non streptococcal upper respiratory tract infection one month after the onset of the disease one patient (R, E) had an exacerbation with increased S_{urea}N for one month.

Two (K, J, A, N) of the four patients classified as IgA GN had increased S_{urea}N early in the course. None of the patients had visible edema but one boy (A, N) aged 4.5 years had a borderline elevation of the blood pressure (170/80). Renin and aldosterone values were slightly elevated in two (P, H, O, M) of the patients at the onset. Moderate proteinuria (max. 2.4–5 g/l) was observed 1.5–2.5 months in three patients (P, H, K, J, A, N) and was still present in one patient (O, M) after 12 months. Recurrences of microscopic hematuria occurred in immediate connection with upper respiratory tract infections and was usually of only a few days' duration. In one patient (O, M) a follow up examination at six months was carried out during an episode of microscopic hematuria. Microscopic hematuria was a constant finding in all patients during the entire observation period. Serum urea N, blood pressure, blood volume, bromide space, renin and aldosterone were consistently within normal limits after the initial phase of the disease.

The patient with non streptococcal proliferative GN had an upper respiratory tract infection one week before the onset of microscopic hematuria. ASO and anti DNase II titers were repeatedly normal. C3 was depressed for about eight months. Oliguria, edema and hypertension were not seen in the early or later stages of the disease. Serum urea N was elevated during the first two months and proteinuria (max. 4.0 g/l) was present for 2.5 months. Blood volume, bromide space, recumbent renin and aldosterone excretion were within normal limits throughout the year. Towards the end of the follow up period urinalysis became normal.

METHODS

The patients were studied in the acute phase and two six and 12 months thereafter. The studies at the first admission were started as soon as possible but were not performed until the S_{urea}N was normal or close to normal. The same protocol was used at each time ex-

cept for renal biopsies which in most cases were performed in the acute phase and one year later.

The patients were placed on a normal diet with 100–140 mEq Na/l/73 m² body surface area (BSA) for at least four days before the study was started. Blood pressure was taken repeatedly over a 24 hour period with an arm cuff and mean values for systolic and diastolic pressures were calculated. Blood volume was determined using a diluting technique with ¹²⁵I albumin. The bromide space was measured with a nuclear activation technique (5). The fasting morning levels of renin (7) and the 4-hour excretion of aldosterone (9–10) were also determined.

Renal biopsy

Percutaneous renal biopsies with a Tru Cut® needle were performed under general anesthesia. No complications occurred. The biopsy material was prepared for examination by light microscopy and immunofluorescence (IFL) using the methods described by Berg et al. (3).

The following fluorescent conjugates were used routinely: heterospecific anti human immunoglobulin (National Bacteriological Lab., Stockholm) and anti human IgG, IgM, IgA, C3 and fibrinogen (Hyland Lab., Costa Mesa, Calif.).

Renal function studies

Standard clearance techniques in conjunction with bladder catheterization were used. The studies were started after at least 17 hours of food and fluid deprivation. Inulin or Inutest and PAH were first administered in a prime dose of 0.4 ml/kg body wt of 45 ml 10% Inulin Laevosan Gesellschaft + 5 ml 20% Sodium aminohippurate MSD or 0.3 ml/kg body wt of 17 ml 25% Inutest Laevosan Gesellschaft + 3 ml 20% Sodium aminohippurate MSD. This was followed by a continuous infusion of the same solution (0.005–0.01 ml/kg body wt/min). After 30 min of equilibration and two control periods of about 10 min a continuous intravenous infusion of Ringer's solution (147 mEq Na/l) was started. The infusion rate was 0.27 ml/kg body wt/min and it was continued until 30 ml/kg body wt or 3% of the body weight had been infused. During saline infusion urine was collected during 15 minute periods. Inulin in blood and urine was estimated by the anthron method (8) and PAH by the method described by Smith et al. (15). Sodium was determined with an Eppendorf flame photometer.

Statistical analysis

Two way analysis of variance was used for testing differences between investigations carried out at different times during the one year follow up. The regression coefficient was calculated and used to measure the increase of sodium excretion during isotonic saline volume expansion. Correlation coefficients were invariably higher than 0.8. From the analysis of variance linear comparisons were made. Data are presented as means \pm SEM.

Five children with previous cystitis but without signs of renal involvement were used as controls for urinary sodium excretion during isotonic saline infusion. Reported findings for GFR, C_{PAH} and filtration fraction (FF) in healthy young adults were used as reference values (1).

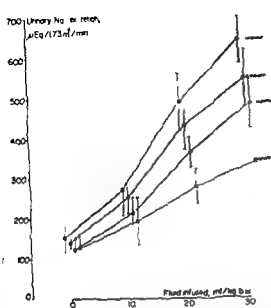


Fig. 3a The urinary Na excretion related to BSA in post streptococcal GN during continuous isotonic saline infusion ($147 \text{ mEq Na/l } 0.3 \text{ ml/kg body wt/min}$ until 3% of the body wt was infused) shortly after the onset of the disease and two, six and 12 months later. Closed circles and bars correspond to means and standard error of the mean (S.E.M.). The shaded area shows the range of values in controls (i.e. children with previous cystitis but without signs of renal involvement).

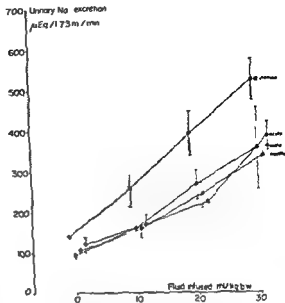


Fig. 3b The urinary Na excretion related to BSA in IgA GN during continuous isotonic saline infusion ($147 \text{ mEq Na/l } 0.3 \text{ ml/kg body wt/min}$ until 3% of the body wt was infused) shortly after the onset of the disease and two, six and 12 months later. Closed circles and bars correspond to means and S.E.M. The shaded area indicates the range of values in control.

creased to normal levels on the last examination. The quotient of GFR and $C_{P_{125}}$ i.e. the filtration fraction (FF) is generally used as an index of the fraction of fluid that is filtered from the plasma passing the glomeruli. Immediately after the onset of the disease the FF during HP in PS GN was decreased and invariably lower than in IgA GN (Table 2). Thereafter the FF was normal in both disorders.

In the patient with non streptococcal proliferative GN the GFR during HP (Fig. 1a) was depressed on the first study shortly after the onset of the disease but it had normalized two months later. After 12 months the GFR on the other hand was again depressed but during VE normalization took place. FF during HP was low on the first investigation as well as 12 months later and VE influenced FF only slightly (Table 2).

Sodium excretion

The renal control of sodium excretion was evaluated by determining the natriuretic response to an isotonic VE with saline. Normally urinary sodium excretion increases in a characteristic manner during saline infusion (see controls in Figs 3a, 3b). The changes in the natriuretic response to VE were similar in PS and IgA GN. Sodium excretion as related to body surface area or glomerular filtration was lower on the first examination than 12 months later (Table 3, Figs 3a, 3b). The differences were significant for both in PS GN ($p < 0.05$) but only for sodium excretion related to body surface area in IgA GN ($p < 0.05$). In PS GN there appeared to be a progressive increase in the capacity to excrete sodium during the year while in IgA GN the capacity to excrete sodium remained depressed at least during the first six months but it then increased to normal levels.

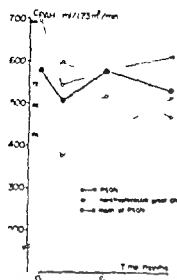


Fig. 2a The C_{cr} during HP in PS and non streptococcal proliferative GN shortly after the onset of the disease and two, six and 12 months later. The means (closed circles) and individual values (open circles) in PS GN (continuous connecting lines) and non streptococcal proliferative GN (interrupted connecting line) are shown.



Fig. 2b The C_{cr} during HP in IgA GN shortly after the onset of the disease and two, six and 12 months later. The means (closed circles) and individual values (open circles) are shown.

The first biopsy from the patient classified as non streptococcal proliferative GN (P A) showed diffuse glomerular hypercellularity. IFL examination was positive only for C3 which was distributed in a granular pattern along the glomerular basement membranes and in the mesangial regions. After 12 months only a slight segmental increase in mesangial matrix remained but IFL examination was unchanged.

Renal function

Hemodynamic studies GFR and C_{cr} were determined at each study during hydropenia (HP) and during isotonic saline volume expansion

(VE). In PS as well as in IgA GN the GFR during HP was depressed and significantly lower immediately following the onset of the disease than on subsequent studies ($p < 0.01$) (Table 1). Serial changes in GFR during HP are illustrated in Fig. 1a and 1b. It is apparent that the major increase in GFR occurred during the first two months of the disease. It is noteworthy that the depression of GFR in acute PS GN as well as in acute IgA GN was abolished by VE (Table 1). PS GN differed from IgA GN with regard to the effect on renal plasma flow. In PS GN C_{cr} during HP (Fig. 2a) was normal on the first examination as well as later. In IgA GN on the other hand the C_{cr} during HP (Fig. 2b) was reduced on the first examination but in

Table 2 Filtration fraction (%) in acute glomerulonephritis

Values are mean \pm S.E.M. HP=hydropenia VE=volume expansion

	Onset		12 months later		Difference onset-12 months later	
	HP	VE	HP	VE	HP	VE
Poststreptococcal GN	12.6 \pm 0.7	14.8 \pm 0.5	19.2 \pm 1.8	23.9 \pm 1.6	+6.6 NS ($p < 0.1$)	+9.1 $p < 0.01$
IgA GN	19.9 \pm 2.5	21.9 \pm 4.9	16.9 \pm 0.9	20.2 \pm 0.5	-3.0 NS	-1.7 NS
Non streptococcal proliferative GN	16.0	15.7	11.7	14.8	-4.3	-0.9

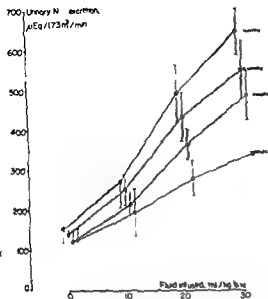


Fig 3a The urinary Na excretion related to BSA in post streptococcal GN during continuous isotonic saline infusion (147 mEq Na/l 0.2 ml/kg body wt/min until 3% of the body wt was infused) shortly after the onset of the disease and two, six and 12 months later. Closed circles and bars correspond to means and standard error of the mean (SEM). The shaded area shows the range of values in control (i.e. children with previous cystitis but without signs of renal involvement).

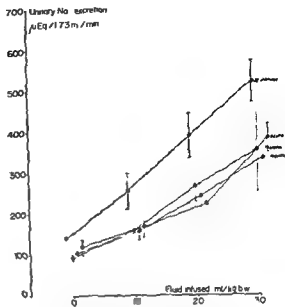


Fig 3b The urinary Na excretion related to BSA in IgA GN during continuous isotonic saline infusion (147 mEq Na/l 0.2 ml/kg body wt/min until 3% of the body wt was infused) shortly after the onset of the disease and two, six and 12 months later. Closed circles and bars correspond to means and SEM. The shaded area indicates the range of values in controls.

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Table 3 Urinary Na excretion ($\mu\text{Eq}/\text{min}$) after volume expansion in acute glomerulonephritis. Values are mean \pm S.E.M. BSA=related to 1.73 m^2 body surface area; GI=related to 100 ml glomerular filtrate

	Onset		12 months later		Difference onset 12 months later	
	BSA	GI	BSA	GI	BSA	GI
Poststreptococcal GN	336.8 ± 1.05	340.3 ± 4.1	638.5 ± 56.7	633.8 ± 42.6	$+301.7$ $P < 0.05^*$	$+293.5$ $P < 0.05^*$
IgA GN	340.8 ± 29.8	367.9 ± 52.8	519.7 ± 90.5	508.8 ± 63.7	$+178.9$ $P < 0.05^*$	$+140.9$ $P < 0.05^*$
Non streptococcal proliferative GN	377.3	411.7	288.1	292.8	-89.2	-118.9

In the patient with non streptococcal proliferative GN however sodium excretion during isotonic saline VF was still depressed 12 months after the onset of the disease (Table 3).

DISCUSSION

Eight patients were admitted to this study. Three had PSGN, four IgA GN and one non streptococcal proliferative GN. Although the series is small it reflects the commonest causes of childhood GN. PS GN is probably the most common type of GN. The incidence of this disease however seems to be declining in Sweden. IgA GN has been reported to account for 12% of biopsied glomerulopathies in children (12). All patients in the present study classified as IgA GN showed the characteristic episodes of macroscopic hematuria in association with upper respiratory tract infections together with IgA predominantly in mesangial regions (12, 13). The first episode of macroscopic hematuria was however more intense than the later ones and in some respects its manifestations closely resembled those of the acute phase of classical GN suggesting that the patients were followed from the onset of their disease.

The pathophysiology of IgA GN resembled in many respects the pathophysiology of PS GN during the first year after the onset of the disease. In both disorders the GFR was decreased at the onset and normal 12 months later. The capacity to excrete sodium was also reduced at the onset of both disorders and

normal 12 months later. The GFR increased to a value that was about 90% of the mean for healthy individuals during hydropnea two months after the onset of both disorders. The early restitution of GFR accords with previously reported determinations of GFR in acute GN (4, 11). It is not clear why the patient with non streptococcal proliferative GN at 12 months follow up had a decreased GFR during HP. The prolonged C₃ depression in diabetes delayed recovery. However morphological improvement occurred and the GFR depression was abolished by VE (Table 1). This would suggest that the depression of GFR was due to temporary functional change rather than irreversible structural changes.

The effect of acute PS GN and IgA GN on effective renal plasma flow differed. In IgA GN the C_{PAH} was low at the onset and the GFR was reduced in proportion to the C_{PAH}. In PS GN on the other hand the C_{PAH} was normal at the onset and the GFR was reduced out of proportion to the C_{PAH}. One might therefore speculate that in acute IgA GN the GFR is reduced because of increased renal vascular resistance while in acute PS GN the GFR is low because of reduced permeability of the glomerular capillary wall. It is of interest in this connection that in IgA GN the IFL-deposits are primarily confined to the mesangium whereas in PS GN they to a greater part are located in or near the capillary basement membrane which might imply a reduced permeability.

Decreased capacity to excrete an intrave-

nous isotonic saline load was observed in all patients with acute GN. The sodium excretion was reduced out of proportion to the glomerular filtration rate which suggests an enhanced tubular sodium reabsorption. Enhanced tubular sodium reabsorption in conjunction with isotonic saline VE has previously been demonstrated in acute experimental GN in dogs (16). Except for slight edema for a few days in one patient with PS GN and slight transient hypertension in one patient with IgA GN, our patients did not have overt signs of salt and water retention and no changes in blood pressure were later seen. Recumbent renin, aldosterone excretion, blood volume and bromide space did not show any characteristic changes during the entire follow up period. It seems likely, however, that the relative inability to increase sodium excretion during VE observed in this study reflects the disturbances which cause the increased extracellular volume that are characteristic of severer forms of acute GN.

All studied parameters of renal function were normal 12 months after the onset of acute PS GN and IgA GN. The late effects of PS GN and IgA GN on renal function can not yet be predicted. A depressed GFR below 70 ml/1.73 m² BSA/min has been found in more than 70% of cases one to 16 years after the onset of PS GN (2). It is therefore possible that a normal GFR 12 months after the onset of PSGN and IgA GN does not reflect a complete recovery but rather a functional adaptation to the disease. It is apparent from this study that renal functional changes do not necessarily parallel renal structural changes and clinical signs of glomerular disease. All patients with IgA GN after one year had clinical signs of active glomerular disease, i.e. microscopic hematuria and episodes of macroscopic hematuria. They also had residual structural glomerular changes and a positive IFL at the end of the follow up period. The biopsy changes were about the same as at the onset. Renal function, however, was within normal limits on the 12 month follow up study. This sug-

gests that renal functional adaptation occurs after the onset of acute GN. If the disease progresses and further structural changes occur, a normal GFR is no longer attainable. Subsequent deterioration in renal function would probably then occur in the course of GN.

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REFERENCES

1. Aperia A, Broberger O, Ericsson N O & Feychting H. Renal function in man during transition from hydropenia to water diuresis with superimposed mannitol load. *Acta Physiol Scand* 77: 479, 1969.
2. Baldwin D M. Poststreptococcal glomerulonephritis: a progressive disease? *Am J Med* 62: 1, 1977.
3. Berg R, Bergstrand A, Bergstrom J, Cunningham R, Tagesson Ch & Wasserman J. Rapidly progressive glomerulonephritis with antibodies against glomerular basement membranes in serum and kidneys. *Clin Nephrol* 5: 37, 1976.
4. Earle D P, Farber S J, Alexander J D & Pellegrino E D. Renal function and electrolyte metabolism in acute glomerulonephritis. *J Clin Invest* 30: 471, 1951.
5. Finley S C & Hare R S. Bromide space in infants and children. *Am J Dis Child* 58: 749, 1959.
6. Gurland H J, Brunner F P, Chantler C, Jacobs C S, Harer A, Selwood N H, Spies G & Wing A J. Combined report on regular dialysis and transplantation in Europe. VI. In B H Robinson P, Vereerstraeten J & B Hawkins (eds). *Proceedings of the European Dialysis and Transplant Association*. Vol. 13. Pitman Press, Bath, 1976, p. 7.
7. Haber E, Koerner T, Page L M, Kliman B & Pernode A. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. *J Clin Endocrinol* 29: 1349, 1969.
8. Hilger H H, Klumper J D & Ullrich A J. Wasserrückresorption und Ionentransport durch die Sammelrohrzellen der Säugetiere. *Pflügers Arch* 267: 18, 1958.
9. Kliman B & Peterson R E. Double isotope denervation assay of aldosterone in biological extracts. *J Biol Chem* 235: 1639, 1960.
10. Langan J, Jackson M, Adin E V & Chantler K B J. A simple radioimmunoassay for urinary aldosterone. *J Clin Endocrinol Metab* 38: 189, 1974.
11. Lewy J, Cruz H & Meicoff J. Acute renal failure and some renal functions in acute glomerulonephritis. In J Meicoff (ed). *Acute glomerulonephritis*. Little Brown & Co, Boston, 1967, p. 339.

- 12 Levy M, Beaufils H, Gubler M C & Habib R. Idiopathic recurrent microscopic hematuria and mesangial IgA-IgG deposits in children (Berger's disease). *Clin Nephrol* 1: 63, 1973.
- 13 McEnery P T, McAdams A J & West C D. Glomerular morphology: natural history and treatment of children with IgA-IgG mesangial nephropathy. In P Kincaid Smith, T H Mathew & F L Becker (eds.) *Glomerulonephritis: Morphology, Natural history and Treatment*. Wiley, New York, 1973, p. 305.
- 14 Schärer K, Chantler C, Brunner F P, Gurland H J, Jacobs C, Selwood N H, Spies G & Wing A J. Combined report on regular dialysis and transplantation of children in Europe. In B H H Robinson P Vereerstraeten & J B Hawkins (eds.) *Proceedings of the European Dialysis and Transplant Association*. Vol. 13. Pitman Press, Bath, 1976, p. 69.
- 15 Smith H W, Finkelstein N, Alimonsa L, Crawford H & Graber M. The renal clearances of substituted hippuric acid derivatives and other aromatic acids in dog and man. *J Clin Invest* 24: 388, 1945.
- 16 Wagnild J P, Gutmann F B & Imb J. Functional adaptation of nephrons in dogs with acute progressing to chronic experimental glomerulonephritis. *J Clin Invest* 57: 1574, 1976.

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STRUCTURAL AND FUNCTIONAL ABNORMALITIES OF THE SMALL INTESTINE IN INFANTS AND YOUNG CHILDREN WITH ROTAVIRUS ENTERITIS

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ABSTRACT Davidson G B and Barnes G L (Department of Gastroenterology, Royal Children's Hospital, Parkville, Victoria, Australia). Structural and functional abnormalities of the small intestine in infants and young children with rotavirus enteritis. *Acta Paediatr Scand* 68 181 1979.—Structural and functional alterations in duodenal mucosa from 17 children with rotavirus enteritis were assessed. Structural changes were found in specimens from all patients. Patients with the most severe mucosal damage were more likely to require intravenous therapy to correct dehydration. Depression of one or more mucosal disaccharidases was found in 14 of 16 patients. Repeat duodenal biopsy three to eight weeks later in six patients showed marked improvement. The study clearly shows that rotavirus can cause a marked structural and functional lesion in the upper small intestine which is rapidly reversible.

KEY WORDS Acute enteritis, children, diarrhoea, disaccharidases, rotavirus, duodenal biopsy.

Several studies have reported the presence of structural abnormalities in duodenal mucosa of children with acute gastroenteritis (3-16). In 1973 studies from this Department showed abnormalities of the duodenal mucosa in 26 of 31 infants with acute gastroenteritis (3). Abnormally low mucosal disaccharidase levels were found in 16 of the infants. A recognized bacterial pathogen was isolated from only one patient. In the remainder it was presumed that the causative agent was a virus although *C. albicans* may have been important in some patients (4).

Later in the year Bishop et al. identified a specific virus as the cause of acute non bacterial enteritis in a group of children admitted to the same hospital (5, 6). This virus has not yet been fully characterized and many names have been assigned to it including orbivirus (6), rotavirus (9), duovirus (8), reovirus like agent (10) and infantile gastroenteritis virus (13). Rotavirus (the name most favoured) is now recognized as a major cause of acute infectious diarrhoea among infants and children throughout the world (14).

We report here structural and functional abnormalities of the duodenal mucosa in children with acute enteritis known rather than assumed to be caused by rotavirus infection.

PATIENTS AND METHODS

Twenty children aged from two to 33 months with acute non bacterial enteritis were examined and had a small bowel biopsy. All were admitted to the Royal Children's Hospital, Melbourne during April to August 1973. Acute enteritis was defined as a febrile illness of less than 10 days duration associated with diarrhoea and vomiting where there was no other evident cause for the symptoms. Rotavirus infection was identified in 17 of these patients (13 males and four females) and they form the subject of this report. Patients one to nine and 11 to 14 in Table 1 were the subjects of earlier reports when rotavirus was first recognized (6, 7).

Duodenal biopsy was performed 4 to 10 hours after onset of symptoms of acute enteritis (Table 1). A repeat biopsy was performed three to eight weeks later in six of the children (patients 3, 4, 8, 9, 17). The biopsy procedure is described in detail elsewhere (17). The biopsy and its possible complications were explained to the parents as reported previously (6). Preparation of biopsy tissue for light and electron microscopy was as described (6). Preparation and examination of faecal specimens by electron microscopy was as previously described (7). Disaccharidase estimations were performed as before (3). Structural changes in duodenal mucosal tissue on light

Table 1. *Structural changes, disaccharidase levels and electron microscope findings in children with rotavirus enteritis*

0=rotavirus negative ++=scanty +++=moderate ++++=profuse NT=not tested

Patient	Age (mo.)	Structural change	Disaccharidases (units/g wet wt.)			Virus in epithelial cells	Virus in faeces	Duration of symptoms before biopsy (hrs.)
			Maltase (normal >9.0)	Sucrase (normal >3.5)	Lactase (normal >1.0)			
1	30	Severe	3.4	0.8	0.2	+++	+++	66
2	33	Severe	2.4	0.7	0.2	0	+	96
3	31	Moderate	2.0	0.3	0	+++	NT	36
4	8	Moderate	NT	NT	NT	+++	+++	77
5	19	Moderate	6.8	1.7	0.4	0	+	96
6	13	Moderate	5.6	1.2	0.3	++	NT	77
7	4	Moderate	10.6	2.7	1.0	+	+++	77
8	8	Moderate	10.1	2.4	2.8	+	+++	170
9	10	Moderate	5.9	1.7	0.4	0	+	170
10	11	Mild to moderate	13.3	4.0	1.7	++	++	96
11	4	Mild	9.9	4.2	0.3	+	+	96
12	14	Mild	2.0	0.3	0.3	++	+	84
13	11	Mild	6.0	1.7	0.9	+	+	74
14	22	Mild	10.0	3.0	1.6	+++	+++	24
15	11	Mild	5.5	1.5	0.9	+	+	96
16	11	Mild	13.1	1.5	1.9	+++	+	77
17	2	Mild	8.1	2.1	0.6	0	+	96

microscopy were graded as showing normal appearance, mild, moderate or severe change as described by Townley et al. (18). Briefly, mild changes included broadening of villi, mild cellular infiltration of the lamina propria and early epithelial cell damage; moderate change involved considerable blunting of villi, obvious increase in inflammatory cells in the lamina propria and epithelial damage; severe change showed complete villous flattening, heavy cellular infiltration and severe epithelial damage.

In 13 of 17 patients rotavirus particles were seen by electron microscopy of ultra thin sections of duodenum and in the other four, rotavirus was identified by electron microscopy of faeces (Table 1).

RESULTS

Structural abnormalities were found in the duodenal mucosa of all patients with rotavirus enteritis. In seven patients the duodenal mucosa was mildly abnormal. Biopsies from eight patients showed moderate damage with blunting of villi, increased crypt depth, flattening of epithelial cells and an increase in inflammatory cells in the lamina propria (Fig. 1). In two patients there were severe changes with loss of villi, crypt hypertrophy, cuboidal epithelium and marked inflammatory infiltrate (Fig. 2).

The appearances were similar to those seen in coeliac disease, although abnormalities tended to be patchy. Electron microscopy of duodenal mucosa from two patients with severe structural change (Fig. 2) revealed marked changes and the presence of rotavirus particles in the cytoplasm (Fig. 3). Three weeks later repeat duodenal biopsy in one of these patients showed normal mucosal structure (Fig. 4) and electron microscopy (Fig. 5).

Disaccharidase levels are listed in Table 1. Depression of one or more enzymes was found in 14 of 16 specimens. The biopsy specimen from patient 4 was too small for measurement of disaccharidase levels. In 10, all three

Fig. 1. Low magnification of biopsy from patient with rotavirus enteritis showing moderate structural damage. Villi are blunted and widened, crypt depth is increased, epithelial cells are abnormal and there is an obvious crease in inflammatory cells in lamina propria (haematoxylin and eosin, $\times 40$).

Fig. 2. Low magnification of section of duodenal mucosa from patient with rotavirus enteritis showing severe structural damage with loss of villi, crypt hypertrophy, epithelial cell damage and marked increase in inflammatory cells in the lamina propria (haematoxylin and eosin, $\times 40$).



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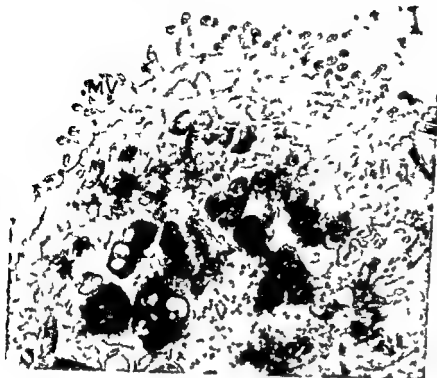


Fig. 3. Electron micrograph of apical portion of epithelial cell from biopsy in Fig. 1. Microvilli (MV) are severely damaged; mitochondria (M) are swollen and numerous virus particles (V) are aggregated with distended endoplasmic reticulum cisternae ($\times 20000$).

zymes were low. Only two patients had normal levels of all disaccharidases.

Six children had a second biopsy. In four of them (patients 2, 3, 9, 12) disaccharidase levels were initially very low, resembling the pattern seen in coeliac disease. To exclude this diagnosis duodenal biopsy was repeated three to eight weeks later. The specimen from patient 3 was too small to allow disaccharidase assay, but duodenal structure had returned to normal. Specimens from the other three patients had normal structure and enzyme levels. Two patients (patients 4 and 8) continued to have diarrhoea following discharge from hospital and a repeat duodenal biopsy was performed to exclude underlying disease. Mucosal structure had recovered but the disaccharidase levels suggested relative sucrose depression (2, 12). Both patients responded symptomatically to a low sucrose diet.

DISCUSSION

Structural and functional alteration of the duodenal mucosa in these 17 children with proven rotavirus enteritis was similar to that reported in our earlier series of 31 children in whom

the cause of the acute enteritis was not identified (3) (Table 2). In retrospect rotavirus was probably responsible for the diarrhoeal illness in most of those children as only one bacterial pathogen was isolated (3).

The damage observed in rotavirus enteritis can be severe enough to be confused with the structural appearance seen in coeliac disease. It appears that the mucosal damage caused by rotavirus can be rapidly repaired as biopsies repeated as early as three weeks after onset of symptoms were normal. The mucosal damage in acute gastroenteritis is quite variable in degree and may be patchy, as noted by others (3). Although the numbers are small, children with a more severe mucosal lesion may be more likely to be dehydrated and require intravenous therapy for rehydration (nine of 10 with moderate or severe damage compared with four of seven with mild structural damage).

Diminished disaccharidase activity in duodenal mucosal homogenates from the majority of patients in this study suggested that sugar malabsorption might have been a significant problem during the acute phase of rotavirus enteritis. This was not so. Stool clintests

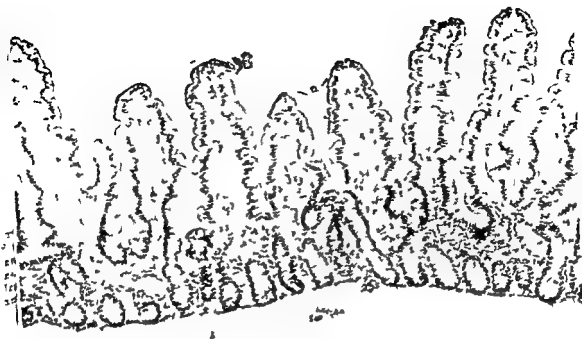


Fig. 4. Low magnification of biopsy from patient shown in Fig. 1 three weeks after recovery from rotavirus enteritis

showing normal duodenal mucosa (haematoxylin and eosin $\times 40$)

were rarely positive (11) but this may be explained by the fact that most infants were drinking only glucose containing fluids at the time of study. The earlier study by Barnes *et al.* (3) suggested that prolonged sugar intolerance was age related and not predictable by acute disaccharidase depression. Only four of the 17 children in the present study were less than six months of age so the majority would not have been expected to exhibit prolonged sugar intolerance.

As rotavirus enteritis can now be effectively diagnosed by faecal electron microscopy or serology it is not likely that many children in the future will have small bowel biopsies during the acute stage of the illness. Structural damage to the upper small bowel mucosa has been described in adults infected with other viruses including the Norwalk (1) and Hawaiian (15) agents, small picorna/parvovirike viruses which to date have not been associated with infectious diarrhoea in infants. Similarly mu-



Fig. 5. Electron micrograph of representative area from biopsy shown in Fig. 4 showing apical portion of two small enterocytes with microvilli junctional complex (JC) and mitochondria (M) ($\times 9000$).

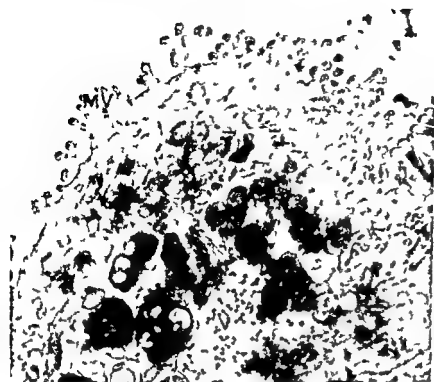


Fig. 3. Electron micrograph of epithelial cell from biopsy in acute gastroenteritis. Microvilli (M) are severely damaged. Mitochondria (M) are swollen and numerous virus particles (V) are aggregated within distended endoplasmic reticulum system ($\times 40000$).

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Diminished disaccharidase activity in duodenal mucosal homogenates from the majority of patients in this study suggested that sugar malabsorption might have been a significant problem during the acute phase of rotavirus enteritis. This was not so. Stool clint-

AGAMMAGLOBULINAEMIA ASSOCIATED WITH THE OCCURRENCE OF A MONOCLONAL IMMUNOGLOBULIN

G F M HENDRICKX B J M ZEGERS and J W STOOP

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ABSTRACT Hendrickx G F M, Zegers B J M and Stoop J W (University Children's Hospital Het Wilhelmina Kinderziekenhuis, Utrecht, The Netherlands). Agammaglobulinaemia associated with the occurrence of a monoclonal immunoglobulin. *Acta Paediatr Scand* 68: 187-191, 1979. A patient is described with a monoclonal immunoglobulin of the IgG class in the serum and no detectable IgM and IgA. Extensive immunological investigations showed the absence of B-lymphocytes in bone marrow and peripheral blood. Moreover, plasma cells were not present in the bone marrow. The monoclonal IgG was synthesized in the gastrointestinal tract. The cellular immune status of the patient was normal. Clinically the patient suffered from gastrointestinal and severe respiratory tract infections. It was concluded that the findings are consistent with the diagnosis congenital agammaglobulinaemia with concurrence of monoclonal IgG. It was postulated that the cell clone in the gastrointestinal tract resulted from an escape of a pre-B cell clone from the recognized arrest of pre-B cells in congenital agammaglobulinaemia.

KEY WORDS Agammaglobulinaemia, monoclonal immunoglobulin

Patients with primary agammaglobulinaemia can be divided into two groups: one with the congenital type and the other with the late-onset type of disease (8, 11). The first of these groups comprises patients with an X-linked recessive genetic pattern or an autosomal recessive pattern as well as isolated cases without a hereditary factor (8). The clinical picture is characterized by an extreme susceptibility to bacterial infections. The serum of these patients contains less than 2 g/l IgG and usually lacks IgA and IgM. No (or very few) immunoglobulin-bearing B-lymphocytes are found in the peripheral blood and no (or very few) plasma cells in the bone marrow (1, 4). Plasma cells have also been reported to be absent in the (intestinal) mucosa of patients with an X-linked agammaglobulinaemia (3).

A distinct genetic pattern is not known for primary acquired agammaglobulinaemia, which is also called late-onset agammaglobulinaemia (8). The signs and symptoms usually appear after the age of ten years. The immunoglobulin levels in the serum are usu-

ally higher than in the congenital forms. B-lymphocytes are present in the peripheral blood and also in the bone marrow, usually in normal numbers (4, 11). Some of the patients show plasma cells in the intestinal mucosa (15). Patients with the late-onset agammaglobulinaemia have not only an increased susceptibility to bacterial infections but also a greater chance of developing autoimmune diseases and malignant processes (11).

The occurrence of monoclonal immunoglobulins in childhood is extremely rare (5). The case reported here concerns a child with a severe immunoglobulin deficiency in whom a monoclonal IgG was demonstrated.

MATERIALS AND METHODS

Humoral and cellular immunity of the patient has essentially been investigated as described in detail earlier (18). Membrane immunofluorescence of the B-lymphocytes and the cytoplasmic immunofluorescence of the plasma cells in the bone marrow has been done as described by Vossen & Hijmans (17). Cytoplasmic immunofluorescence of the jejunal and rectal biopsies was performed as described by Mul et al. (16). The anal

Table 2 Comparison of mucosal structure and disaccharidase levels from two groups of children with acute gastroenteritis

	Barnes et al 1973 (1) (unknown aetiology)	Present study (rotavirus enteritis)
Mucosal structural changes		
Normal	5	0
Mild change	11	8
Moderate change	10	7
Severe change	5	2
Disaccharidase depression (1 or more)	16	14
Total patients	31	17

Disaccharidase levels not measured in patient 4

cosyl disaccharidase depression a prominent feature in rotavirus enteritis has also been described in other viral enteritides (1-15). This report documents structural and functional changes in small bowel mucosa in a group of children known to be suffering from rotavirus enteritis.

ACKNOWLEDGEMENTS

The authors acknowledge the support and encouragement of Dr R R W Townley whose patients we studied and whose initiative prompted this work. We also thank Dr Ruth F Bishop who conceived the idea of applying the technique of electron microscopy to the study of gastroenteritis which ultimately led to the discovery of the rotavirus. We thank Mr Brian Ruck for the electron micrographs, Mr J Smith for the light microscopy photographs, Mrs R Kay for preparation of the histological sections and Mr Max Murray for disaccharidase assays.

REFERENCES

- Agus S G, Dolin M, Wyatt R G, Tousimis A J & Northrup R S. Acute infectious non bacterial gastroenteritis: intestinal histopathology. Histologic and enzymatic alterations during illness produced by the Norwalk agent in man. *Ann Intern Med* 79: 111-117, 1973.
- Ament M E, Perera D R & Esther L J. Sucrase isomaltase deficiency—a frequently misdiagnosed disease. *J Pediatr* 83: 721, 1973.
- Barnes G L & Townley R R W. Duodenal mucosal damage in 31 infants with gastroenteritis. *Arch Dis Child* 48: 343, 1973.
- Bishop R F, Barnes G L & Townley R R W. Microbial flora of stomach and small intestine in infantile gastroenteritis. *Acta Paediatr Scand* 62: 40, 1974.
- Bishop R F, Davidson G P, Holmes I H & Ruck B J. Evidence for viral gastroenteritis. *VE J Med* 759: 1096, 1973.
- Bishop R F, Davidson G P, Holmes I H & Ruck B J. Virus particles in epithelial cells of duodenal mucosa from children with acute non bacterial gastroenteritis. *Lancet* II: 1241, 1973.
- Bishop R F, Davidson G P, Holmes I H & Ruck B J. Detection of a new virus by electron microscopy of faecal extracts from children with acute gastroenteritis. *Lancet* I: 149, 1974.
- Davidson G P, Bishop R F, Townley R R W, Holmes I H & Ruck B J. Importance of a new virus in acute sporadic enteritis in children. *Lancet* 247: 1974.
- Flawell T H, Bryden A S, Davies H W & G N Binder J C & Derrick J M. Relationship between viruses from acute gastroenteritis of children and newborn calves. *Lancet* II: 61, 1974.
- Harikari A Z, Kim H W, Wyatt R G, Rugez W J, Cline W L, Parrott R H & Chonock E M. Reoviruslike agent in stools. Association with infantile diarrhoea and development of serological tests. *Science* 195: 1049, 1974.
- Kerry K R & Anderson C M. A ward test for sugar in faeces. *Lancet* I: 981, 1964.
- Kerry K R & Townley R R W. Genetic pattern of intestinal sucrase isomaltase deficiency. *Paediatr J* 1: 223, 1965.
- Martin M J, Palmer E L & Middleton P J. Ultrastructure of infantile gastroenteritis virus. *Virology* 68: 146, 1975.
- Rotavirus of man and animals. *Lancet* I: 757, 1974.
- Schreiber D S, Bicklow N R & Trier J S. The small intestinal lesion induced by Hawaiian acute infectious non bacterial gastroenteritis. *J Infect Dis* 129: 705, 1974.
- Toccolino H, Licastro R, Cardo A G & Williams M. Histologic changes in the small intestine in children with acute diarrhoea. *J Paediatr* 78: 90, 1971.
- Townley R R W & Barnes G L. Intestinal biopsy in childhood. *Arch Dis Child* 48: 480, 1973.
- Townley R R W, Khaw K T & Shwachman H. Quantitative assay of disaccharidase activities in small intestinal mucosal biopsy specimens in infant and childhood. *Pediatrics* 36: 911, 1965.

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normal (62%). In vitro stimulation with phytohaemagglutinin (PHA) pokeweed mitogen (PWM) and tetanus toxoid (an anamnestic antigen) gave normal ^{14}C thymidine incorporation. In mixed lymphocyte culture (MLC) experiments a normal stimulator and responder capacity was observed. PWM stimulation of the patient's lymphocytes gave good lymphocyte proliferation as judged from the normal ^{14}C thymidine incorporation but blasts containing immunoglobulin were not demonstrable (9).

CLINICAL COURSE

As already mentioned the *Giardia lamblia* infection was successfully treated before the investigations mentioned above. After the studies gammaglobulin substitution was started (initial dose 1.4 ml/kg of a 16% solution followed by a monthly maintenance dose of 0.7 ml/kg). The infection of the respiratory tract was treated with an antibiotic (amoxycillin). Moderate swelling of the mucosa of the sinus maxillare persisted. The physical and radiological signs in the lungs largely disappeared. The lung infection improved. Prophylaxis with antibiotics was however required because symptoms and signs of bronchopneumonic infection reappeared after the amoxycillin was withdrawn. The malabsorption was still present to the same degree six months after the treatment was started and also after the disappearance of the *Giardia lamblia*. The increase in height and weight were normal and there were no signs of nutritional deficits. During this treatment the serum IgG decreased to a level lower than 1.7 g/l as determined in serum obtained prior to each gammaglobulin injection. The jejunum biopsy specimen taken after six months of treatment showed fewer plasma cells, the villous atrophy was less pronounced and the lactate content ($9\text{ }\mu\text{mol/g wet weight}$) lay within normal limits.

At present the patient has been followed for two years and his condition is still good with an occasional exacerbation of the respiratory tract infection which responded well to a therapeutic dose of cotrimoxazol.

DISCUSSION

The clinical picture of this patient is consistent with a specific humoral defence disturbance. The quantitative determination of the serum immunoglobulins (Ig) showed an IgG level that was not distinctly anomalous for the patient's age but IgM and IgA were not present. This kind of serum Ig pattern is usually considered to indicate dysimmunoglobulinaemia. Qualita-

tive analysis of the IgG showed that it was monoclonal. Specific antibodies were not demonstrable in the serum. B lymphocytes were not present in the peripheral blood and neither these lymphocytes nor Ig containing plasma cells were present in the bone marrow. The specific cellular immunity was intact. Thus the findings in this case are more consistent with the diagnosis primary agammaglobulinaemia with—as a very striking feature—occurrence of monoclonal IgG.

In children monoclonal Ig—sometimes transient—have been demonstrated for instance in some cases of (severe) combined immunodeficiency (4, 19, 24), secondary immunodeficiency and malignant processes of the lymphoreticular system (21, 22, 23), coeliac disease (18), the haemolytic uraemic syndrome (25), chromosomal abnormalities and congenital infections (4, 17). Immunological reconstitution after bone marrow transplantation is often accompanied by the appearance in the serum of monoclonal Ig which are transient and reflect the maturation of the B cell system (20). To the best of our knowledge the clinical picture of multiple myeloma has never been described in childhood (12) and is also not to be expected in this age group (13). No indications pointing to multiple myeloma were found in our patient since the radiological investigation of the skeleton and the bone scintigraphy showed no anomalies; the bone marrow was morphologically normal except for the absence of plasma cells. Bence Jones protein was not found and there was no calciumuria. The production of monoclonal IgG was extra medullary and was localized in the gastrointestinal tract as shown by immunofluorescence studies in mucosal biopsy specimens. Consequently the possibility of an extra-osseous myeloma or extra medullary plasmacytoma localized in the gastrointestinal tract could not be ruled out. In these patients however abdominal complaints usually predominate but our patient did not have such complaints and neither the clinical nor the radiological investigation pro-

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CASE REPORT

R. I. was born at term after an uneventful pregnancy. Diphtheria, pertussis, tetanus, poliomyelitis and small pox vaccinations did not lead to unusual reactions. At the age of one and a half years a severe infection of the respiratory tract required hospitalization and frequent recurrences were accompanied by a high fever. Chicken pox and measles ran in uncomplicated course but mumps gave rise to a very severe herpetic infection of the upper lip in association with a stomatitis ulcerosa. Quantitative determination of the serum immunoglobulins in this connection (at the age of five years) showed that neither IgA nor IgM were present in the serum. The IgG content was 4.11 g/l. Agar gel and immuno electrophoresis were not performed. At the age of seven the child was referred to our hospital because of increasingly copious purulent sputum and suspicion of bronchiectasis. The height and weight lay at 190 and 170 for the age respectively. Rhinitis mucopurulenta was observed and on auscultation of the lungs diffusely spread rhonchi and crepitations were heard in both lower lobes. The teeth were missing. The physical examination showed no other anomalies.

The sedimentation rate of the erythrocytes was 25 mm. The haemoglobin and haematocrit values were normal. The blood count showed leucocytosis ($21.3 \times 10^9/l$), the differential count showed 1% eosinophils, 2% basophils, 4% bandforms, 79% segmented neutrophils and 14% lymphocytes. Normal serum levels were found for electrolytes, calcium, inorganic phosphate, alkaline phosphatase, urea, creatinine, transaminases, cholesterol and uric acid. The calcium content of the urine was normal. The sweat test gave normal results. The blood group was O rhesus positive. The total haemolytic complement content was elevated.

Radiological investigation of the skeleton and bone scintigraphy showed no anomalies. X-rays of the nasal sinuses showed sinusitis maxillaris and the thorax X-ray showed infiltrative changes in both lower lobes. At bronchography the right middle lobe bronchus was found to be narrowed with poor filling of the peripheral region. The branches of the right lower lobe bronchus showed irregular borders.

The lung function investigation showed a slightly reduced vital capacity i.e. 1540 ml (normal for height 1800 ml). Culture of the nasal secretion and the sputum yielded an abundance of *Haemophilus influenzae*.

The faeces showed *Giardia lamblia* which was treated with metronidazole before functional investigation of the gastrointestinal tract. Fat excretion was normal. Nitrogen

excretion was however elevated (1.47 g/24 h normal <1 g/24 h) (14). This could be partially ascribed to a protein loss since in the $Cr^{51}Cl_2$ test the total excretion of the marker over a 4-day period amounted to 9.0% (normal <1%). D-xylose absorption was normal and lactose uptake was reduced. The jejunum biopsy specimen showed moderate and in some places even total atrophy of the villi as well as signs of chronic enteritis. The lactase level in the biopsy specimen was low (0.9 $\mu\text{mol/g}$ wet weight). The levels of sucrose isomaltase and maltase were normal.

IMMUNOLOGICAL INVESTIGATIONS

Humoral immunity

Agar gel electrophoresis of the serum showed a small homogeneous band in the gamma globulin region on immuno electrophoresis with specific antisera; this band proved to be IgG1 type λ . IgG of other subclasses or type κ was only present in very small amounts. The serum IgG content was 5.67 g/l. IgM and IgA were not demonstrable and the same holds for isohaemagglutinins, antistaphylococcal, antistreptococcal and antibodies against the mumps and measles viruses. Antibodies against diphtheria, tetanus and poliomyelitis were also undemonstrable even after a booster injection of DTP vaccine. No immunoglobulin bearing B lymphocytes were present in the peripheral blood or bone marrow and no immunoglobulin containing plasma cells were found in the latter. Plasma cells found in the biopsy specimens from the jejunum and rectum contained IgG type λ but plasma cells with IgA or IgM were not present. In the nasal secretion IgG of type λ was found but not IgA or IgM. The urine did not contain Bence Jones protein.

Cellular immunity

The number of lymphocytes in the peripheral blood was always more than $2.0 \times 10^9/l$. Delayed type hypersensitivity to mumps was present (induration 15 mm). After sensitization with dinitrochlorobenzene (DNCB) the challenge led to a positive reaction. The percentage of T lymphocytes (E rosettes) was

normal (62%). In vitro stimulation with phyto haemagglutinin (PHA) pokeweed mitogen (PWM) and tetanus toxoid (an anamnestic antigen) gave normal ^{14}C thymidine incorporation. In mixed lymphocyte culture (MLC) experiments a normal stimulator and responder capacity was observed. PWM stimulation of the patient's lymphocytes gave good lymphocyte proliferation as judged from the normal ^{14}C thymidine incorporation but blasts containing immunoglobulin were not demonstrable (9).

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At present the patient has been followed for two years and his condition is still good with an occasional exacerbation of the respiratory tract infection which responded well to a therapeutic dosage of cotrimoxazol.

DISCUSSION

The clinical picture of this patient is consistent with a specific humoral defence disturbance. The quantitative determination of the serum immunoglobulins (Ig) showed an IgG level that was not distinctly anomalous for the patient's age but IgM and IgA were not present. This kind of serum Ig pattern is usually considered to indicate dysimmunoglobulinaemia. Qualita-

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At present the patient has been followed for two years and his condition is still good with an occasional exacerbation of the respiratory tract infection which responded well to a therapeutic dosage of cefotriaxol.

DISCUSSION

The clinical picture of this patient is consistent with a specific humoral defence disturbance. The quantitative determination of the serum immunoglobulins (Ig) showed an IgG level that was not distinctly anomalous for the patient's age but IgM and IgA were not present. This Ig pattern is usually considered agammaglobulinaemia. Qualita-

tive analysis of the IgG showed that it was monoclonal. Specific antibodies were not demonstrable in the serum. B lymphocytes were not present in the peripheral blood and neither these lymphocytes nor Ig-containing plasma cells were present in the bone marrow. The specific cellular immunity was intact. Thus the findings in this case are more consistent with the diagnosis primary agammaglobulinaemia with—as a very striking feature—occurrence of monoclonal IgG.

In children monoclonal Ig—sometimes transient—have been demonstrated for instance in some cases of (severe) combined immunodeficiency (4, 19, 24), secondary immunodeficiency and malignant processes of the lymphoreticular system (21, 22, 23), coeliac disease (18), the haemolytic uraemic syndrome (25), chromosomal abnormalities and congenital infections (4, 17). Immunological reconstitution after bone marrow transplantation is often accompanied by the appearance in the serum of monoclonal Ig which are transient and reflect the maturation of the B cell system (20). To the best of our knowledge the clinical picture of multiple myeloma has never been described in childhood (12) and is also not to be expected in this age group (13). No indications pointing to multiple myeloma were found in our patient since the radiological investigation of the skeleton and the bone scintigraphy showed no anomalies; the bone marrow was morphologically normal except for the absence of plasma cells. Bence Jones protein was not found and there was no calcium. The production of monoclonal IgG was extra-medullary and was localized in the gastrointestinal tract as shown by immunofluorescence studies in mucosal biopsy specimens. Consequently the possibility of an extra-osseous myeloma or extra-medullary plasmacytoma localized in the gastrointestinal tract could not be ruled out. In these patients however abdominal complaints usually predominate but our patient did not have such complaints and neither the clinical nor the radiological investigation pro-

ysis of immunoglobulin containing blasts after culture of the lymphocytes with pokeweed mitogen (PWM) has been performed as published by Gmelig Meyling et al (9). The combined xylose-lactose tolerance test has been done as described by Fernandez & Van de Kamer (7). Gastrointestinal protein loss has been investigated by using intravenously administered $\text{Cr}^{51}\text{Cl}_3$. The small intestinal disaccharidases were investigated as described by Van Eggemont & Hers (6).

CASE REPORT

R. L. was born at term after an uneventful pregnancy. Diphtheria, pertussis, tetanus, poliomyelitis and small pox vaccinations did not lead to unusual reactions. At the age of one and a half years a severe infection of the respiratory tract required hospitalization and frequent recurrences were accompanied by a high fever. Chicken pox and measles ran an uncomplicated course but mumps gave rise to a very severe herpetiform infection of the upper lip in association with a stomatitis ulcerosa. Quantitative determination of the serum immunoglobulins in this connection (at the age of five years) showed that neither IgA nor IgM were present in the serum. The IgG content was 5.31 g/l (Aptel) and immuno electrophoresis were not performed. At the age of seven the child was referred to our hospital because of increasingly copious purulent sputum and suspicion of bronchiectasis. The height and weight lay at P90 and P70 for the age respectively. Rhinitis mucopurulenta was observed and auscultation of the lungs diffusely spread rhonchi and crepitations were heard in both lower lobes. The teeth were cinous. The physical examination showed no other anomalies.

The sedimentation rate of the erythrocytes was 24 mm. The hemoglobin and hematocrit values were normal. The blood count showed leucocytosis ($21.3 \times 10^9/l$), the differential count showed 1% eosinophils, 2% basophils, 4% bandforms, 79% segmental neutrophils and 14% lymphocytes. Normal serum levels were found for electrolytes, calcium, inorganic phosphate, alkaline phosphatase, urea, creatinine, transaminases, cholesterol and uric acid. The calcium content of the urine was normal. The sweat test gave normal results. The blood group was 0 rhesus positive. The total hemolytic complement content was elevated.

Radiological investigation of the skeleton and bone scintigraphy showed no anomalies. X rays of the nasal sinuses showed sinusitis maxillaris and the thorax X ray showed infiltrative changes in both lower lobes. At bronchography the right middle lobe bronchus was found to be narrowed with poor filling of the peripheral region. The branches of the right lower lobe bronchus showed irregular borders.

The lung function investigation showed a slightly reduced vital capacity (11550 ml (normal for height 18000 ml). Culture of the nasal secretion and the sputum yielded an abundance of *Haemophilus influenzae*.

The faeces showed *Giardia lamblia* which was treated with metronidazole before functional investigation of the gastrointestinal tract. Fat excretion was normal. Nitrogen

excretion was however elevated (1.41 g/24 h normal $<1 \text{ g/24 h}$) (14). This could be partially ascribed to a protein loss since in the $\text{Cr}^{51}\text{Cl}_3$ test the total excretion of the marker over a 4-day period amounted to 100% (normal $<1\%$). D-xylose absorption was normal and lactose uptake was reduced. The jejunum biopsy specimen showed moderate and in some places even total atrophy of the villi as well as signs of chronic enteritis. The lactase level in the biopsy specimen was low ($0.4 \mu\text{mol/g wet weight}$). The levels of sucrase, maltase and maltase were normal.

IMMUNOLOGICAL INVESTIGATIONS

Humoral immunity

Agar gel electrophoresis of the serum showed a small homogeneous band in the gamma globulin region on immuno electrophoresis with specific antisera this band proved to be IgG1 type λ . IgG of other subclasses or type μ was only present in very small amounts. The serum IgG content was 5.67 g/l. IgM and IgA were not demonstrable and the same holds for isohaemagglutinins, antistaphylococcal, antistreptolysin and antibodies against the mumps and measles viruses. Antibodies against diphtheria, tetanus and poliomyelitis were also undemonstrable even after a booster injection of DTP vaccine. No immunoglobulin bearing B lymphocytes were present in the peripheral blood or bone marrow and no immunoglobulin containing plasma cells were found in the latter. Plasma cells found in the biopsy specimens from the jejunum and rectum contained IgG type λ but plasma cells with IgA or IgM were not present. In the nasal secretion IgG of type λ was found but not IgA or IgM. The urine did not contain Bence Jones protein.

Cellular immunity

The number of lymphocytes in the peripheral blood was always more than $2.0 \times 10^9/l$. Delayed type hypersensitivity to mumps was present (induration 15 mm). After sensitization with dinitrochlorobenzene (DNCB) the challenge led to a positive reaction. The percentage of T lymphocytes (E rosettes) was

- macytoma with intracellular fibrils *Am J Gastroenterol* 64 00 1975
- 11 Hermans P E Diaz Buxo J A & Stobo J E Idiopathic late-onset immunoglobulin deficiency. Clinical observations in 50 patients *Am J Med* 61 1 1876
- 12 Hewell G M & Alexanian R Multiple myeloma in young persons *Ann Intern Med* 84 441 1976
- 13 Hobbs J R Immunocytoma of mice and men *Br Med J* 11 67 1971
- 14 Kamer J H van de & Wauters E A K. Mal absorption *Schweiz Med Wschr* 106 285 1976
- 15 Matuchansky C Galian A Goldfarb D Ram biud J C Modigliani R & Bernier J J Immunocytes III immunoglobulines du tube digestif au cours des defits immunitaires primitifs de l'adulte. Etude de 6 cas *Castr enterol Clin Biol* 1 331 1977
- 16 Mul N A J de Boer L E M Tammeau J A & Balheux R E Light chain type ratio of IgA producing cells in human jejunal biopsies *Eur J Clin Invest* 5 61 1975
- 17 Oxelius V A Monoclonal immunoglobulins in congenital toxoplasmosis *Clin Exp Immunol* 11 367 1977
- 18 Pena A S Nieuwkoop J Schuyt H R E Hekkens W Th J M & Haer A J Ch Transient paraproteinaemia in a patient with coeliac disease *G I* 17 735 1976
- 19 Rudi J Dooren L J Morell A Skviri F Vos en J M J J & Uittenbogaart C H Immunoglobulins and transient paraproteins in sera of patients with the Wiskott Aldrich syndrome: a follow up study *Clin Exp Immunol* 1 25 756 1976
- 20 Rudi J Dooren L J Eysvoegel V P Went J J van & Hymans W An immunological study during post transplantation follow up of a case of severe combined immunodeficiency *Clin Exp Immunol* 1 10 367 1977
- 21 Solomon A Homogeneous (monoclonal) immunoglobulin in cancer *Am J Med* 63 169 1977
- 22 Stoop J W Balheux R E & Weyers H A Paraproteinaemia with secondary immunoglobulin deficiency in an infant *Pediatrics* 79 97 196
- 23 Stoop J W Zegers B J M van der Heiden C & Balheux R E Monoclonal gammopathy in a child with leukemia *Blood* 37 774 1968
- 24 Stoop J W Eysvoegel V P Zegers B J M Blok Schut Ph D van Bekkum H W & Balheux R E Selective severe cellular immunodeficiency effect of thymus transplantation and transfer factor administration *Clin Immunol Immunopathol* 6 289 19 6
- 25 Vlist G J van der Donckerwolcke H A Huyten R H & Zegers B J M The haemolytic uraemic syndrome associated with *Yersinia enterocolitica* infection and transient paraproteinaemia (in preparation)
- 26 Vogler L H Gathings W E Lawton A H & Cooper M E Immunofluorescent studies of the development of pre B cells B lymphocytes and immunoglobulin isotype diversity in humans *Eur J Immunol* 7 804 1977
- 27 Vossen J M & Hymans W Membrane associated determinants on bone marrow and blood lymphocytes in the pediatric age group and on fetal tissues *Ann NY Acad Sci* 754 67 1975
- 28 Zegers B J M Maertzdorf W J van Loghem E Mul N A J Stoop J W van der Laag J Vossen J M & Balheux R E Kappa chain deficiency an immunoglobulin disorder *Engl J Med* 94 10 1976

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vided indications suggesting a tumour in the gastrointestinal tract. Moreover the morphology of the plasma cells in the intestinal biopsy specimen was normal. There are few follow up reports on this kind of patients and relatively little is known about the course of though a rapidly fatal development has been documented in a few cases (10). Our patient showed a steady clinical improvement which also makes a malignant plasmacytoma unlikely.

The presence of monoclonal IgG in a patient lacking mature B cells is intriguing. Recently the occurrence of precursor B cells in patients with a congenital agammaglobulinemia whose bone marrow showed neither B lymphocytes nor plasma cells was reported (26). On this basis it is conceivable that in our patient a pre B cell clone might have escaped the arrest of the pre B cells in congenital agammaglobulinemia (26). It is not impossible that intense stimulation at the level of the gut for instance by bacterial lipopolysaccharides led to the development of the cell clone found there. Broom et al (3) postulated a similar mechanism to explain the presence of plasma cells in the jejunum of patients with late onset agammaglobulinemia.

In our patient antibiotic therapy and gamma globulin injections led to considerable improvement of the clinical condition. A striking feature was the associated decrease of monoclonal IgG in the serum. Although we can only speculate this observation suggests that the treatment inhibited the elusive stimulus for synthesis. The decrease of the IgG in the serum was accompanied by a decrease in the density of the plasma cells in the jejunum and an improvement of the villous pattern and the enzyme activity. However these last two findings could be purely coincidental because it is highly improbable that successive biopsy specimens are taken from the same place (15).

With respect to the prognosis it can be said that humoral immunodeficiency responds well to treatment. The activity of the cell clone in

the intestinal mucosa can be followed rather easily on the basis of quantitative serum IgG determinations performed in serum sampled before successive gammaglobulin injections. Whether degeneration of the clone will occur remains to be seen.

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REFERENCES

1. Abdou N I, Casella S R, Abdou N L & Abrahamson J A. Comparative study of bone marrow and blood B cells in infantile and acquired agammaglobulinemia. *J Clin Invest* 57: 2218 (1973).
2. Ament M E, Ochs H D & Davis S D. Structure and function of the gastrointestinal tract in primary immunodeficiency syndromes. A study of 39 patients. *Medicine* 52: 227 (1973).
3. Broom B C, De la Concha E G, Webster A D, B. Loeur G & Asherson G L. Dichotomy between immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinemia. *Lancet* II 243 (1975).
4. Cooper M D, Lawton A R & Bockman D E. Agammaglobulinemia with B lymphocytes: specific defect of plasma cell differentiation. *Lancet* II 791 (1971).
5. Danon F & Seligmann M. Serum monoclonal immunoglobulins in childhood. *Arch Dis Child* 48: 207 (1973).
6. Eggermont E van & Hers H G. The sedimentation properties of the intestinal α glucosidases of normal human subjects and of patients with sucrose intolerance. *Eur J Biochem* 9: 488 (1969).
7. Ferrnides I & van de Kamer J H. The combined xylose disaccharide tolerance test: its application for diagnosing disaccharidase deficiency. *Acta Paediatr Scand* 60: 187 (1971).
8. Gatti R A. On the classification of patients with primary immunodeficiency disorders. *Clin Immunol Immunopathol* 3: 243 (1974).
9. Gmelig Meyling F H J, UytdeHaag A G C M & Ballicux E E. Human B cell activation in vitro. T cell dependent pokeweed mitogen induced differentiation of blood B lymphocytes. *Cell Immunol* 33: 156 (1977).
10. Harper T B, Powers J M, Russell H E, Dunn W B & Gardner W A. Primary small bowel plas-

A SURVEY OF 164 FINNISH CHILDREN AND ADOLESCENTS WITH HYPERTENSION

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ABSTRACT Uhari M and Koskimies O (Department of Paediatrics University of Oulu and Children's Hospital University of Helsinki) A survey of 164 Finnish children and adolescents with hypertension Acta Paediatr Scand 68 193 1979 — A retrospective analysis was performed on 164 children and adolescents with persistent hypertension Among the unselected 115 patients with hypertension seen within the last three years 47 (41%) exhibited renal disease 37 (32%) coarctation of the aorta 10 (9%) miscellaneous associated causes and 11 (18%) no associated cause (essential hypertension) A substantial number 53/164 had a primary disease potentially curable by surgery and in 37 patients the blood pressure was normalized postoperatively The outcome depended mostly on the basic disease and the availability of chronic hemodialysis 11/164 children have died all because of terminal basic disease and one with simultaneous hypertensive crisis We thus recommend a thorough investigation in the case of a child with persistent hypertension

KEY WORDS Children adolescents hypertension blood pressure

In most published series severe hypertension in childhood is found to be mainly secondary in nature most often due to renal diseases or coarctation of the aorta (4 17 20) Gill et al suggest that essential hypertension is rare in children finding only one case in their series of 100 British children with hypertension (4) Little information is available from other paediatric centres in Europe but in the United States the prevalence of essential hypertension has been found to be in general higher (1 9 12 15) even up to 95% in one series of mildly hypertensive children (14)

We present here a retrospective analysis of 164 hypertensive children and adolescents examined and followed up in two University Hospitals in Finland The purpose was to find out the main causes of hypertension in childhood and the percentage of children with no detectable primary cause of their hypertension

PATIENTS AND METHODS

Patients The study was based on a total of 164 children with hypertension who had been treated and followed up

in the Department of Paediatrics University of Oulu and in the Children's Hospital University of Helsinki 115 of these children had visited the hospitals during the period 1973-1975 and the remaining 49 had been diagnosed and followed up earlier

Criteria for hypertension The following criteria were used for inclusion a diastolic pressure of 90 mmHg or more or a systolic pressure of 130 mmHg or more in children under 5 years or a systolic pressure of 140 mmHg or more in older children all measured repeatedly over 3 months or more These values are well above those published for normal Finnish children (18) The authors are aware that many infants and children with mild or labile hypertension or deficient follow-up are excluded under these criteria (14)

Investigations performed The type of investigation was related to the initial clinical evaluation or diagnosis If necessary one or more of the following examinations were carried out aortography renal angiography cardiac catheterization renal vein renin samples or renal biopsy Before making the final diagnosis of essential hypertension the primary causes of hypertension were excluded by renal function studies urine analyses for bacteria ECG chest roentgenogram intravenous urography hormone estimations and in some cases renal angiography and renin estimations ()

RESULTS

The age distribution of the 164 patients 63 girls and 101 boys is seen in Fig 1 The mean blood pressure recorded was 163/107 mmHg

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RESULTS

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Table 2 *Diagnoses associated with hypertension by age groups*

Only patients followed up during the years 1973-1975 are representative for calculating the percentage distribution of the causes of hypertension

Diagnosis	I Patients followed up during 1973-1975				II Other patients	Total
	Number of patients by age groups					
	0-5	5-10	10-16	Total (%)		
Renal disease				47 (41%)	36	83
Chronic glomerular disease	1	4	7	12	15	
Pyelonephritis				9	6	
With reflux		4	3			
Without reflux		1	1			
Obstructive uropathy	1	3	3	7	2	
Polycystic kidneys	3	1	1	5	1	
Renovascular disease	1	2	1	4	2	
Dysplastic kidneys	4			4		
Nephronophthisis		1	1	2	2	
Nephropathia		1	1	2	5	
Interstitial nephritis		1		1	3	
Hemolytic uremic syndrome		1		1		
Coarctation of the aorta	13	14	10	37 (32%)	2	39
Arterio-sclerosis juvenilis		1	1	2	1	3
Turner's syndrome		1	1	2	1	3
Phaeochromocytoma			1	1	2	3
Nephroblastoma	1			1	1	2
Leukaemia (renal damage)		1		1	1	2
Pseudohypoparathyreosis			1	1		1
Obesity				2		2
Essential hypertension				21 (18%)	6	27
Obscure	1	2	9	12		
Suspected			7	9		
Total				115	49	164

The total of 164 patients contained 13 in which no reason could be found for the elevated blood pressure: renal arteriography being normal. These patients fulfilled the criteria of essential hypertension (2). A further 14 patients were suspected to have essential

Table 3 *Outcome in 164 hypertensive patients followed up for 1-17 years (mean 4 years)*

Outcome	No. of patients
Cured (no longer receiving treatment)	
By operation	37
Weight loss	1
Under medication	47
Under observation	43
Deceased after transplantation	11
Successful renal transplantation	6
Hemodialysis after unsuccessful transplantation	2
Hemodialysis (and medication)	2
Not known	4
Total	164

hypertension since no obvious primary disease was detected but as renal arteriography had not been performed the diagnosis could not be established with certainty. Eight of these children had labile hypertension with their blood pressure elevated for three months or more but recorded as normal on some occasions afterwards. Essential hypertension occurred in 21 (18%) of the 115 patients. Obesity was the only finding in two children with hypertension.

Treatment and outcome The follow up time varied from one year to 17 years with a mean of 4 years. 59 children received continuous medical treatment and 43 were followed up untreated (Table 3). The latter included 6 with coarctation of the aorta waiting for an operation at the time of compiling the data. Diuretics were the most common antihypertensive preparation used and methyl dopa, reserpine, clonidine and hydralazine were also used. The

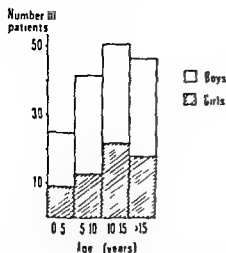


Fig. 1 Distribution of the 164 hypertensive children by sex and age at the time of the survey

Reasons for referral 48 of the children had been referred to hospitals because of elevated blood pressure including 17 who were symptomless and were identified at routine screening in a Child Welfare Centre or in a school health examination. The other children had symptoms including cardiac murmur, proteinuria, urinary tract infection or cardiac insufficiency (Table 1).

Causes of hypertension The numerical and percentage distributions of the conditions associated with hypertension in the patients followed up during the period 1960–1975 are depicted in Table 2. Since cardiological patients could not be identified accurately from the earlier years, as seen in Table 2, the patients examined during the years 1973–1975 are thus documented separately.

The most common cause of hypertension in these 115 cases was found to be renal disease. Chronic glomerular disease was found in 12 children, four with primary and eight with secondary glomerular disease. The latter ones had the following main diagnoses: three patients had anaphylactoid purpura, three had collagen disease, one had hydrocephalus and shunt nephritis and one had Alport's syndrome. In the category of pyelonephritis seven patients had moderate to severe vesicoureteral reflux (Grades III–V) and two had no reflux at all at the time when hypertension

was discovered (6). The diagnosis of pyelonephritis was based on repeated positive urine culture results and pathological findings at intravenous urography, voiding cystography, renal angiography or renal biopsy. One child had interstitial nephritis due to prolonged phenacetin ingestion. The earlier part of the series included one boy with interstitial nephritis due to hypercalcaemia from vitamin D overdosage.

One of the patients listed under nephropathy had renal biopsy changes that were not clearly classifiable and another had longstanding juvenile diabetes mellitus with renal damage. Renal damage developed in one patient during the induction treatment for leukemia. One of the two patients with nephroblastoma was first detected due to a hypertensive crisis while the other developed hypertension at the age of 15, having been in good health for ten years after the treatment of the primary tumour and metastases in the lung with surgery and radiotherapy.

Concretization of the aorta was the most frequently observed single condition causing hypertension being present in a total of 37 children (32%). Three patients also had a vascular lesion: severe atherosclerosis and systolic hypertension. These cases are reported elsewhere (8).

Table 1 Main clinical symptoms or signs leading to admission of the hypertensive children

Symptom or sign	No of patients
High blood pressure with other symptom	31
Cardiac murmur	21
Proteinuria	18
High blood pressure without other symptom	17
Urinary tract infection	17
Cardiac insufficiency	11
Hematuria	8
Headache	7
Convulsions	6
Anemia	4
Growth retardation	3
Others (palpable tumour, renal insuff. etc.)	21
Total	164

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Remedily used (and medication)	4
Not known	4
Total	164

Treatment and outcome The follow up time varied from one year to 17 years with a mean of 4 years. 59 children received continuous medical treatment and 43 were followed up untreated (Table 3). The latter included 6 with coarctation of the aorta waiting for an operation at the time of compiling the data. Diuretics were the most common antihypertensive preparation used and methyldopa, reserpine, clonidine and hydralazine were also used. The

Table 4 Types and outcome of surgical intervention in 64 hypertensive children

Diagnosis	Operation	No of patients	BP normalized in
Coarctation of the aorta	Reconstruction	35	26
Unilateral renal disease	Nephrectomy	6	6
Reflux nephropathy	Antireflux operation	4	
Phaeochromocytoma	Excision of tumour	3	2
Renovascular disease	Arterial reconstruction	2	
	Nephrectomy	1	1
Renal insufficiency	Transplantation	10	
Obstructive uropathy	Resection of urethral valve	1	
Shunt nephritis	Removal of the shunt	1	
Total		64	37

medical treatment was effective in all cases of essential hypertension.

A total of 64 patients had been operated upon mainly for reconstruction of the aorta (Table 4). Out of the total of 164 children the blood pressure of 38 was completely normalized, 37 after surgical treatment and one after losing weight (Table 3). Ten children underwent renal transplantation of which there were four rejected.

There were eight children with hypertensive crisis which were successfully treated. Nine children died during the follow-up because of a renal disease (Table 3), the primary disease being pyelonephritis in three of these cases and glomerular disease in the others. Hypertension became worse in these cases as renal insufficiency advanced. One child died of nephroblastoma and hypertensive crisis and one of those with severe arteriosclerosis died during the follow-up period.

DISCUSSION

Hypertension is an important factor when considering morbidity and mortality in adults and it is a cause of disease and even death in childhood and infancy (11, 13, 17). Childhood hypertension and blood pressure are of interest because of the possibility that they may be markers of future vascular complications (1, 22).

It has been claimed that childhood hyperten-

sion is not so rare as it has been found to occur with a prevalence of 1.4–8.2% in children and adolescents (3, 12, 15). Of course hypertension can be defined in quite different ways, e.g. statistically (those individuals exceeding the mean by 2 S.D. or more) or by empirical determined numerical blood pressure value, e.g. 140/90 mmHg. Thus the prevalence quoted are not always comparable (7). Our material was selective from this point of view and no prevalence figure can be calculated.

The measurement of blood pressure as routine part of the physical examination does not yet seem to work well in Finland since only approximately every fourth child had his/her blood pressure measured by the primary care physician before referral to hospital. Even so, 17 children had been referred precisely because their blood pressure was found to be high in routine health screening.

The same etiological factors can cause hypertension in children and adults (16, 17) but most authors claim that the possibilities of finding a curable cause for the hypertension are clearly greater in children than in adults (4). This has been challenged by some, however (14). Our series, although somewhat selective, clearly showed that in properly investigated patients secondary hypertension was found in more than 80% of all hypertensive children and adolescents.

The most common cause of hypertension in younger patients was coarctation of the aorta.

(Table 2) The proportion of cases with renal disease was greater among the older children. This is understandable as patients with coarctation are cured early whereas most acquired glomerular diseases first develop at school age and need time to progress before the appearance of hypertension.

Pylonephritis was associated with hypertension in 9 (8%) of the 115 patients. If hypertension is caused by pylonephritis and vesicoureteral reflux there should be renal damage present (13). However Schapiro demonstrated experimentally in rats that hypertension can increase the susceptibility to pylonephritis (19). It is possible that our two cases of pylonephritis without renal damage first had hypertension and later developed pylonephritis.

Although renal disease and coarctation of the aorta were the main causes of hypertension, essential hypertension was identified in 18% of our patients. It is probable that the proportion of essential hypertension would be greater in an epidemiological study (1, 10, 21). This contrasts with the results of Gill et al. whose study represented highly selected cardiac and renal patients with an even higher mean blood pressure (4).

The outcome for children with hypertension depends first of all on the basic disease (20). Patients with hypertension due to an incurable disease fare better when the hypertension is controlled using antihypertensive medication and even renal function might improve (9). The attitude of paediatricians to the treatment of hypertension has been conservative which explains our finding that 43 patients with obvious hypertension were placed under observation without treatment. All the children who died during the follow-up period suffered from severe incurable disease but as in the other cases their hypertension could be kept under control by medication or surgical intervention.

Since 53 out of the 164 patients had surgically curable disease coarctation, renovascular disease or unilateral renal disease patients with hypertension should be thoroughly

investigated. Thus we completely disagree with authors who advocate a passive attitude to the examination of children with hypertension (14).

The even more difficult and still unsolved question is how to screen for hypertension in children and particularly in infants. The best available screening method would include the routine measurement of blood pressure, careful palpation of femoral pulses and urine analysis, all of which should be a part of a normal child welfare examination.

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REFERENCES

1. Aschberg L, C. Zeis P, M. Miller R, A. John E, G. & Chan L. Essential hypertension in childhood. *JAMA* 238: 37, 1977.
2. Barrat T, M. Hypertension in childhood. In: Williams (ed.) *Urology in childhood*. Encyclopedia of Urology, Suppl. XV. Springer Verlag, New York, 1974, p. 34.
3. Cassimos C, H. Varlamis G, Karampous S, & Katsouyannopoulos V. Blood pressure in children and adolescents. *Acta Paediatr Scand* 66: 439, 1977.
4. Gill D, G. daCosta B, M. Cameron J, S. Joseph M, C. Ogg C, S. & Chantler C. Analysis of 100 children with severe and persistent hypertension. *Arch Dis Child* 51: 951, 1976.
5. Haggerty B, J. Maroney M, W. & Nadas A, S. Essential hypertension in infancy and childhood. Differential diagnosis and therapy. *Am J Dis Child* 97: 535, 1956.
6. Heikel P, E. & Parkkainen M, V. Vesico-ureteric reflux in children. A classification and results of conservative treatment. *Ann Radiol* 9: 1, 1966.
7. Julius B. Classification of hypertension. In: Genest H, Korw. & O. Kuchel (eds.) *Hypertension*. McGraw-Hill, New York, 1977, p. 9.
8. Kautla I, Rapola J, Selonen A, Lappalainen J, & Joki T. A new type of severe arteriosclerosis beginning in childhood. *Symposium on clinical genetics*. Oslo, 1977.
9. Kincaid Smith P. Renal disease and hypertension. *Med Clin North Am* 61: 611, 1977.
10. Lieberman E. Essential hypertension in children and youth. A pediatric perspective. *J Pediatr* 85: 1, 1974.
11. Lundquist A, & Wallgren G. Unilateral renal artery stenosis and fatal arterial hypertension in a newborn infant. *Acta Paediatr Scand* 51: 575, 1962.
12. Loggie J, M. H. Prevalence of hypertension and distribution of causes. In: M. I. New & L. S. Levine

- (eds) *Juvenile hypertension* Kroc Foundation series vol 8 Raven Press New York 1977 p 1
- 13 Loggie J M Systemic hypertension in children and adolescents Causes and treatment *Pediatr Clin North Am* 18 1273 1971
- 14 Londe S Goldring D Gollub S W & Hernandez A Blood pressure and hypertension in children Studies problems and perspectives In M I New & I Levine (eds) *Juvenile hypertension* Kroc Foundation series vol 8 Raven Press New York 1977 p 13
- 15 Masland R Heald F Goodale W & Gallagher J Hypertensive vascular disease in adolescence *New Engl J Med* 255 894 1956
- 16 Pickering G *Hypertension Causes consequences and management* Churchill Livingstone Edinburgh 1974
- 17 Rance C P Arbus G S Baffe J W & Koob S W Persistent systemic hypertension in infants and children *Pediatr Clin North Am* 21 801 1974
- 18 Rasanen L Ahlstrom A & Kantero E L National survey of Finnish rural children *Ann Acad Sci Fennicae A I Medica* 168 1 1975
- 19 Shapiro A Experimental pyelonephritis and hypertension Implications for the clinical problem *Ann Med* 59 37 1963
- 20 Still J L & Cottom D Severe hypertension childhood *Arch Dis Child* 49 313 1974
- 21 Uhari M Unpublished data
- 22 Zimmer S H Martin L F Sacks F Rosner B Kass E A longitudinal study of blood pressure childhood *Am J Epidemiol* 100 437 1975

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FREE AND BOUND TRYPTOPHAN IN HUMAN PLASMA DURING THE PERINATAL PERIOD

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ABSTRACT Tricklebank M B, Pickard F J and De Souza S W (Department of Child Health, University of Manchester, Manchester, England). Free and bound tryptophan in human plasma during the perinatal period. *Acta Paediatr Scand* 68: 199-204, 1979.—The concentration of tryptophan and the degree of binding of the amino acid to protein were examined in human plasma during the perinatal period. Both total and unbound (free) tryptophan were higher in cord vein plasma than in the maternal circulation, the concentration gradient being approximately 1:2. The proportion of the total plasma tryptophan concentration that was not bound to protein was less in cord vein plasma than in the maternal circulation. After birth the proportion in infant plasma fell significantly. Both total and free tryptophan fell during the first 24 hours of postnatal life. Total tryptophan returned to the cord vein plasma level 6-8 days after birth whilst free tryptophan failed to increase during the period of the observations. In premature infants total and free tryptophan also declined in concentration 12-24 hours after birth, suggesting the phenomenon to be related to birth rather than to gestational age. Phenylalanine remained unchanged whilst tyrosine increased in concentration during the first 80 hours of postnatal life. Thus the availability of tryptophan to the tissues appears to decline during the immediate postnatal period and the results suggest that the requirement for tryptophan during this time may exceed the supply from standard artificial milk preparations.

KEY WORDS Human neonate plasma, free bound tryptophan

Tryptophan is unique amongst the plasma amino acids in that in both human and rodent blood between 80% and 90% circulates bound to serum albumin (13). Factors influencing tryptophan albumin binding have received much attention recently since apart from the role of the amino acid in protein synthesis the availability of tryptophan is an important factor in the control of the synthesis of the neurotransmitter 5-hydroxytryptamine in brain (9, 12). The binding of tryptophan to albumin is susceptible to disturbance by many factors. Thus the intake of food (23, 29), stressful manipulation (18) and the administration of certain pharmacological agents (15) may increase the concentration of free tryptophan in plasma; increases in brain tryptophan and 5-hydroxytryptamine turnover often occurring concomitantly (7, 15, 18). Disturbances in the disposition of tryptophan in plasma occur in a number of neuropathological

states (3, 6, 30) and there is some evidence that the subsequent alteration in brain tryptophan metabolism may have functional consequences (34).

A notable exception to the apparent necessity for maintaining this control over plasma tryptophan exists in the neonatal rat. Over 90% of the total plasma tryptophan concentration has been found to be in the free form for up to 60 hours after birth (4, 5). Thereafter binding to albumin increases such that only about 40% is free around 5 days of age (4, 33). The high proportion of tryptophan in the free form in the plasma of newborn animals is associated with both high brain tryptophan concentration and a synthesis rate of 5-hydroxytryptamine close to its maximal value (4).

It is not known however whether similar changes in the disposition of tryptophan occur in human plasma during the perinatal period. Studies of the maternal, fetal and neonatal

Table 1 Total and free tryptophan in maternal and cord vein plasma following induced or spontaneous labour

Mean values ± 1 S.D. of 8 samples are shown. The significance of differences were assessed by one way analysis of variance followed by paired comparisons *t* test

	Tryptophan (nmol/ml)		% free tryptophan
	Total	Free	
Maternal plasma 1—before induction of labour	43.31 \pm 8.48	12.18 \pm 3.91	22.94 \pm 4.99
Maternal plasma 2—induced labour well established	48.74 \pm 11.55	12.93 \pm 4.71	26.83 \pm 8.35
Cord plasma	101.47 \pm 27.24	20.46 \pm 6.27	18.39 \pm 5.50
Significance of differences of cord plasma values from maternal samples 1 and 2 (<i>p</i>)	<0.001	<0.001	<0.05
Cord plasma—spontaneous labour	111.07 \pm 25.09	19.68 \pm 5.00	14.57 \pm 4.83

plasma aminogram have tended to exclude tryptophan often for methodological reasons (19–20, 21–37) and as a consequence little is known of the factors that may influence the availability of tryptophan to the tissues in early life. This information may be of clinical significance since there is evidence that insults such as perinatal asphyxia and early under nutrition may alter plasma tryptophan and cerebral 5-hydroxytryptamine metabolism (16–33) changes which may be important for subsequent neuronal development. In the present study the binding of tryptophan to albumin in human plasma has been examined during the immediate postnatal period and an attempt has been made to determine the extent to which the disposition of tryptophan in plasma may be influenced by events or procedures peculiar to the perinatal period.

METHODS

The sample included infants born by normal vertex vaginal delivery following spontaneous labour or labour induced by rupture of membranes and infusion of oxytocin (Syn-tocinon, Sandoz Products Ltd, London, U.K.). All babies were breathing spontaneously by 1 min after birth. The gestational ages of premature babies ranged from 28 to 36 weeks and that of term babies from 37 to 43 weeks. The birth weights were appropriate for gestational age (26). All babies received their first milk feed (SMA, Wyeth Laboratories, Mordenhead, U.K.) by 3 hours of age, 90 ml/kg body weight in the first 24 hours, 120 and 150 ml/kg on the second and third days respectively and 180 ml/kg

on subsequent days. During the period of study all subjects were devoid of clinical abnormalities.

Blood was collected from mothers by venipuncture before the onset of and during established labour from the umbilical cord vein at birth and from babies by heel stab (using minimum pressure) at various times after birth. Unless stated otherwise only one sample of blood was obtained from any one infant. All samples were obtained with the informed consent of the mothers.

Blood samples were mixed with heparin (approximate 11.3 mg/ml blood) and stored at 4°C in sealed containers for less than 24 hours before centrifugation at 1000 \times g for 10 min at room temperature. Plasma was withdrawn and immediately stored at -20°C. After thawing free and bound tryptophan in plasma were separated by ultrafiltration as previously described (18) except that samples were centrifuged at 450 \times g for 10 min at 37°C. Tryptophan was determined in plasma (total tryptophan) and in the plasma ultrafiltrate (free tryptophan) by the method of Denckla & Dewey (11). Tyrosine and phenylalanine were determined in plasma by the methods of Waalkes & Udenfriend (136) and McCammon & Robins (74) respectively.

Recovery of known amounts of the amino acids added to plasma was between 90% and 100%. Duplicates differed by less than 5%. In the study of the effects of postnatal age on plasma amino acid levels each assay contained at least 3 cord plasma samples and a balanced representation of samples from the range of postnatal age considered. Storage of blood for 24 hours at 4°C prior to the separation of plasma had a negligible effect on the results.

RESULTS

Total and free tryptophan in maternal and cord plasma

The total and free concentration of tryptophan in cord plasma did not differ significantly

Table 2 Total and free tryptophan in plasma from premature infants

Mean values \pm 1 S.D. of 6 samples are shown. The significance of differences was assessed by the paired-comparisons test.

	Tryptophan (nmol/ml)		Free tryptophan
	Total	Free	
Cord plasma	89.13 \pm 11.01	11.55 \pm 4.52	12.93 \pm 4.77
Plasma from blood taken 17-24 hours after birth	48.34 \pm 17.88	6.96 \pm 7.78	12.20 \pm 4.60
Significance of differences (p)	<0.01	<0.02	N.S.

N.S. = not significant

between infants born following spontaneous or induced labour (Table 1). Total and free tryptophan in cord plasma were however 208% and 198% respectively of the concentration in the maternal circulation sampled when induced

labour was well established (Table 1). In cord plasma about 18% of the total tryptophan concentration was in the free form significantly less than the percentage in the maternal circulation both before (22%) and after (26%) the onset of labour (percent free tryptophan, Table 1). There was no significant difference between the two maternal samples with respect to total tryptophan, free tryptophan or the percent free tryptophan (Table 1).

Total and free tryptophan in plasma during the neonatal period

After birth the percentage of free tryptophan declined significantly from $18 \pm 4\%$ (mean \pm 1 S.D.) to $12 \pm 3\%$ by 12 hours after birth ($p < 0.005$, Fig. 1). More dramatically both the total and free concentrations of tryptophan fell rapidly after birth. Total tryptophan was only 50% of the cord value in samples collected between 7 and 24 hours of postnatal age (Fig. 1). Thereafter there appeared to be a gradual recovery and 6-8 days after birth the concentration was not significantly different from that of cord blood. During the first 11 hours of life free tryptophan was highly variable but by 7-12 hours after birth it was only 42% of the cord level. Unlike total tryptophan free tryptophan remained low throughout the duration of the subsequent observations.

Total and free tryptophan in plasma from premature infants

Plasma total and free tryptophan were determined in cord blood from premature infants

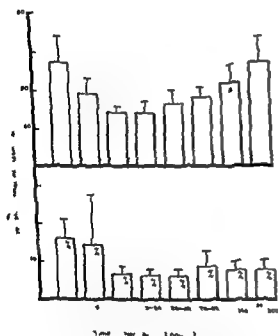


Fig. 1 Total and free tryptophan in human cord blood plasma and in heel prick samples taken at various times after birth. Bars represent 1 S.D. Numbers in upper histogram represent the number of samples in each group. Percentages refer to the percentages of the plasma total tryptophan concentration that was not bound to protein (percent free tryptophan). The significance of differences were assessed by analysis of variance followed by *t* test. Significantly different from cord vein plasma, $p < 0.001$. Significantly different from samples from subjects less than 6 hours old, $p < 0.01$.

Table 1 *Total and free tryptophan in maternal and cord vein plasma following induced or spontaneous labour*

Mean values ± 1 S.D. of 8 samples are shown. The significance of differences were assessed by one way analysis of variance followed by paired comparisons *t* test.

	Tryptophan (nmol/ml)		<i>f</i> _c free tryptophan
	Total	Free	
Maternal plasma 1—before induction of labour	53.31 \pm 8.49	12.18 \pm 3.91	22.54 \pm 4.99
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	Tryptophan (nmol/ml)		% free tryptophan
	Total	Free	
Cord plasma	89.13 \pm 11.01	11.55 \pm 4.5	12.93 \pm 4.77
Plasma from blood taken 1-24 hours after birth	58.34 \pm 1.88	6.96 \pm 0.78	12.70 \pm 4.60
Significance of differences (<i>p</i>)	<0.01	<0.01	N.S.

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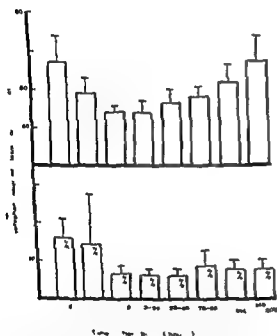


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(32) The decline in both total and free tryptophan concentration after birth could reflect the sudden removal of the placental supply route without concomitant adjustment of the uptake and utilisation of the amino acid by the tissues. However, a similar decline in plasma tryptophan concentration occurs in the rat although in this species the decline does not commence until the animal is at least 30 hours old (4). An alternative explanation—that of induction of metabolism along the pyrrolase pathway—is unlikely since the concentration of kynurenine and its metabolites is very low in neonatal urine even after ingestion of a tryptophan load (1, 38). The phenomenon is at least related to birth rather than to developmental age since a significant fall of both total and free tryptophan was found to occur in premature infants. As tryptophan is an essential amino acid it is possible that artificial milk feed preparations (given within 3 hours of birth) are deficient in tryptophan. In this respect it is of interest that plasma phenylalanine levels remained essentially unchanged whilst tyrosine steadily increased. Feeding experimental animals a diet deficient in tryptophan rapidly depletes both the plasma and the brain of the amino acid (2) and induces a number of behavioural abnormalities probably mediated through decreased formation of 5-hydroxytryptamine (14, 22). The present results therefore suggest that more attention should be given to the investigation of the supply and utilisation of tryptophan by the human neonate.

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REFERENCES

1. Aumcchio S, Quagliarielli M & Rubino A. On the presence of metabolites of the tryptophan-nicotinic acid chain in the urine of sucklings during the first month of life. *Paediatr Acta* 15: 479 (1960).
2. Bopp M, Fadd F, Fanni P, Tagliamonte A & Gessa G L. Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindole acetic acid by a tryptophan free diet. *Life Sci* 14: 131 (1974).
3. Bloxam D L & Curzon G. A study of the proposed determinants of brain tryptophan concentration after portacaval anastomosis or sham-operation. *J Neurochem* (in press).
4. Bourgoin S, Faivre Bauman A, Benda P, Glowinski J & Hamon M. Plasma tryptophan and 5-HT metabolism in the CNS of the newborn rat. *J Neurochem* 23: 319 (1974).
5. Bourgoin S, Faivre Bauman A, Héry F, Terneux J P & Hamon M. Characteristics of tryptophan binding in the serum of the newborn rat. *Biol Neonate* 31: 141 (1977).
6. Coppen A, Eccleston E G & Peet M. Plasma tryptophan binding in depression. *Adv Biochem Psychopharmacol* 11: 375 (1974).
7. Curzon G & Fernando J C R. Effect of aminophylline on tryptophan and other aromatic amino acids in plasma, brain and other tissues and on brain 5-hydroxytryptamine metabolism. *Br J Pharmacol* 48: 533 (1976).
8. Curzon G, Friedel J & Knott P J. The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature* 242: 198 (1973).
9. Curzon G, Joseph M H & Knott P J. Effects of immobilisation and food deprivation on rat brain tryptophan metabolism. *J Neurochem* 19: 1967 (1972).
10. Curzon G & Knott P J. Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acid in the plasma. *Br J Pharmacol* 40: 197 (1974).
11. Denckla W D & Dewey H K. The determination of tryptophan in plasma, liver and urine. *J Lab Clin Med* 69: 166 (1967).
12. Fernstrom J D & Wurtman R J. Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* 173: 149 (1971).
13. Fuller R W & Roush D W. Binding of tryptophan to plasma proteins in several species. *Comp Biochem Physiol* 46B: 273 (1973).
14. Gallup G G Jr, Wallman L B, Boren J L, Gallagher G J, Maser J D & Edson P H. Tryptophan and tonic immobility in chickens: effects of dietary and systemic manipulations. *J Comp Physiol Psychol* 91: 647 (1977).
15. Gessa G L & Tagliamonte A. Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis. *Adv Biochem Psychopharmacol* 11: 119 (1974).
16. Kalyandaram S. Effect of dietary protein and caloric deficiency on tryptophan levels in the developing rat brain. *J Neurochem* 27: 1745 (1976).
17. Keefe D K, Kay J L, Brown B E & Nordquist B. Plasma FFA and blood sugar in mothers and their newborn infants. *Pediatrics* 37: 597 (1966).
18. Knott P J & Curzon G. Free tryptophan in plasma and brain tryptophan metabolism. *Nature* 239: 455 (1977).
19. Lindblad B S. The venous plasma free amino acid levels during the first hours of life. I. After normal and

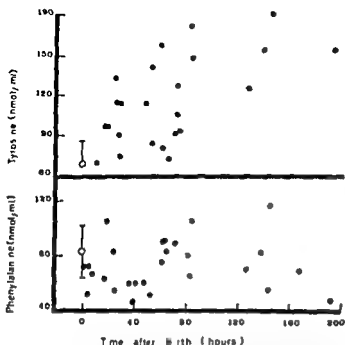


Fig. 2 Tyrosine and phenylalanine in infant blood plasma at various times after birth. Values at time 0 were obtained from cord vein blood and the accompanying bars indicate 1 S.D. about the mean value obtained. There were 13 and 25 cord vein plasma samples assayed for tyrosine and phenylalanine respectively.

and in the same infants 12–24 hours of age were 66% and 60% of the cord plasma values respectively. Unlike the immediate postnatal changes seen in full term infants, the decline in plasma tryptophan was not accompanied by a significant alteration in the percentage of tryptophan not bound to protein (Table 2).

Tyrosine and phenylalanine in plasma during the neonatal period

No consistent pattern of change in concentration of phenylalanine was found to occur in plasma during the neonatal period whilst plasma tyrosine levels steadily increased (Fig. 2).

DISCUSSION

It is well known that the total concentration of α amino nitrogen is higher in cord blood than in the maternal circulation. Individual amino acids vary in their concentration gradients across the placenta from about 1.2 to 1.4, there being little in this respect to differentiate

the essential from the non essential acids (20–37). Tryptophan does not appear to be an exception to this rule, the concentration gradient of both total and free forms being about 1.7.

Significantly less tryptophan was found to be free in cord blood than in the maternal circulation. Furthermore, the proportion increased during the first 12 hours of postnatal life, after which only about 12% was free, though this is probably an under estimate because the separation did not take place at physiological pH and the loss of Co from the sample during preparation and storage favored a rise in pH and the enhancement of binding (25). The results are nevertheless in marked contrast with the newborn rat in which following similar separation procedures more than 80% of the total plasma tryptophan is bound (4, 5).

It has been suggested that the lack of binding of tryptophan to albumin in the rat may be due, at least in part, to high levels of circulating non-esterified fatty acids (NEFA) during the neonatal period (4, 5). NEFA compete with tryptophan for binding to albumin (8) and increased lipolysis is associated with increased free tryptophan levels (10, 23). The high degree of binding in human cord plasma and the subsequent enhancement following birth may therefore be anomalous since plasma NEFA's are reported to rise markedly during the first 24 hours of postnatal life (17, 27, 35).

A further anomaly perhaps exists in the maternal circulation: there was no significant difference in the concentration of total or free tryptophan, or in the percentage free tryptophan between samples taken immediately before the onset of labour and when labour was well established. NEFA's again probably increased markedly during this time (31). Hence the binding of tryptophan would be expected to decrease (8, 18). This finding may be of interest in view of the recent evidence suggesting an association between disturbances in tryptophan albumin binding and the onset of depressive symptoms in women following childbirth.

(32) The decline in both total and free tryptophan concentration after birth could reflect the sudden removal of the placental supply route without concomitant adjustment of the uptake and utilisation of the amino acid by the tissues. However a similar decline in plasma tryptophan concentration occurs in the rat although in this species the decline does not commence until the animal is at least 30 hours old (4). An alternative explanation that of induction of metabolism along the pyrrolase pathway is unlikely since the concentration of kynurenine and its metabolites is very low in neonatal urine even after ingestion of a tryptophan load (1, 38). The phenomenon is at least related to birth rather than to developmental age since a significant fall of both total and free tryptophan was found to occur in premature infants. As tryptophan is an essential amino acid it is possible that artificial milk feed preparations (given within 3 hours of birth) are deficient in tryptophan. In this respect it is of interest that plasma phenylalanine levels remained essentially unchanged whilst tyrosine steadily increased. Feeding experimental animals a diet deficient in tryptophan rapidly depletes both the plasma and the brain of the amino acid (2) and induces a number of behavioural abnormalities probably mediated through decreased formation of 5-hydroxytryptamine (14, 22). The present results therefore suggest that more attention should be given to the investigation of the supply and utilisation of tryptophan by the human neonate.

ACKNOWLEDGEMENTS

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REFERENCES

- Aunuchio S, Quagliariello E & Rubino A. On the presence of metabolites of the tryptophan nicotinic acid chain in the urine of sucklings during the first month of life. *Helv Paediat Acta* 15: 479, 1960.
- Biggio G, Fadda F, Fanni M, Tagliamonte A & Gessa G L. Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindole acetic acid by a tryptophan free diet. *Life Sci* 14: 1371, 1974.
- Blotman D L & Curzon G. A study of the proposed determinants of brain tryptophan concentration after portacaval anastomosis or sham-operation. *J Neurochem* (in press).
- Bourgoignie J, Favre Bauman A, Benda P, Glowinski J & Hamon M. Plasma tryptophan and 5-HT metabolism in the CNS of the newborn rat. *J Neurochem* 23: 319, 1974.
- Bourgoignie S, Favre Bauman A, Héry F, Terneux J P & Hamon M. Characteristics of tryptophan binding in the serum of the newborn rat. *Biol Neonate* 31: 141, 1977.
- Coppen A, Eccleston E G & Peet M. Plasma tryptophan binding in depression. *Adv Biochem Psychopharmacol* 11: 375, 1974.
- Curzon G & Fernando J C R. Effect of aminopyrrolone on tryptophan and other aromatic amino acids in plasma, brain and other tissues and on brain 5-hydroxytryptamine metabolism. *Br J Pharmacol* 58: 533, 1976.
- Curzon G, Friedel J & Knott P J. The effect of fatty acids on the binding of tryptophan to plasma protein. *Nature* 247: 198, 1973.
- Curzon G, Joseph M H & Knott P J. Effects of immobilisation and food deprivation on rat brain tryptophan metabolism. *J Neurochem* 19: 1967, 1977.
- Curzon G & Knott P J. Effects on plasma and brain tryptophan in the rat of drugs and hormones that influence the concentration of unesterified fatty acid in the plasma. *Br J Pharmacol* 50: 197, 1974.
- Denicola W D & Dewey H K. The determination of tryptophan in plasma, liver and urine. *J Lab Clin Med* 69: 160, 1967.
- Fernstrom J D & Wurtman R J. Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* 173: 149, 1971.
- Fuller R W & Roush B W. Binding of tryptophan to plasma proteins in several species. *Comp Biochem Physiol* 46B: 773, 1973.
- Gallop G G, Jr, Wallnau L B, Boren J L, Gallagher G J, Maser J H & Edson P H. Tryptophan and tonic immobility in chickens: effects of dietary and systemic manipulations. *J Comp Physiol Psychol* 91: 647, 1977.
- Gessa G L & Tagliamonte A. Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis. *Adv Biochem Psychopharmacol* 11: 119, 1974.
- Kalyansundaram S. Effect of dietary protein and caloric deficiency on tryptophan levels in the developing rat brain. *J Neurochem* 27: 1745, 1976.
- Keele D K, Kay J L, Brown H S & Nordquist H. Plasma FFA and blood sugar in mothers and their newborn infants. *Pediatrics* 37: 597, 1966.
- Knott P J & Curzon G. Free tryptophan in plasma and brain tryptophan metabolism. *Nature* 239: 457, 1972.
- Lundblad H S. The venous plasma free amino acid levels during the first hours of life. 1. After normal and

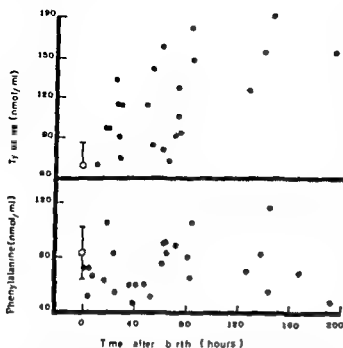


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17 HYDROXYPROGESTERONE IN NORMAL CHILDREN AND CONGENITAL ADRENAL HYPERPLASIA

Measurement in Serum by Radioimmunoassay after Thin layer Chromatography

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ABSTRACT Petersen K. F. and Christensen T. (The Children's Hospital Fuglebakken, University of Copenhagen and Medi Lab, Copenhagen, Denmark). 17 hydroxyprogesterone in normal children and congenital adrenal hyperplasia. *Acta Paediatr Scand* 68: 205, 1979. Serum 17 α hydroxyprogesterone (17 OH P) was measured by a specific radioimmunoassay technique combined with thin layer chromatography. Normal values for children are <1.1 μ g/l (<3.3 nmol/l)—corresponding to values found in the literature. In congenital adrenal hyperplasia (CAH) values up to several hundred μ g/l are found. The values rise after ACTH stimulation and are suppressed by dexamethasone or cortisone treatment. The rise in 17 ketosteroids and pregnanetriol in untreated CAH is relatively smaller (15–25 fold). This clinical sensitivity of 17 OH P is thus valuable for the diagnosis of CAH (21 hydroxylase deficiency). Furthermore it is easier to take a blood sample than to collect urine for 24 hours. The usefulness in therapeutic monitoring is being studied.

KEY WORDS 17 α hydroxyprogesterone, radioimmunoassay, thin layer chromatography, children, congenital adrenal hyperplasia.

Measurement of serum 17 α hydroxyprogesterone (17 OH P) is of interest in congenital adrenal hyperplasia (CAH). Defective 21 hydroxylation is found in the majority (up to 95%) of the patients with the syndrome.

17 OH P accumulates and the blood concentration will rise several hundred fold. There is a (relatively smaller) rise in the urinary excretion of pregnanetriol. Cortisol in plasma is lowered and the negative feedback mechanism leads to further ACTH stimulation of the early steps in the biosynthetic pathway. The increased production of androgens will result in a higher excretion of 17 ketosteroids (17 KS) in the urine. Diagnosis and therapeutic monitoring of cortisone treatment in CAH rests on the clinical parameters: height, growth acceleration, virilization and bone age development—until recently supplemented by examination of the excretion of total 17 KS and pregnanetriol in the urine. In the search for a

more sensitive indicator for diagnosis and treatment control and to try to avoid the collection of urine, we were interested in developing and evaluating the use of serum 17 OH P.

METHODS

17 hydroxyprogesterone method

Reagents: Buffer: 0.01 M sodium phosphate, 0.15 M sodium chloride, 0.1% (w/v) sodium azide, 0.1 (w/v) gelatine, 0.05% (w/v) bovine gamma globulin, pH 7.2.

17 H 17 α hydroxyprogesterone: 40–60 Ci/mole (New England Nuclear) was purified by thin layer chromatography prior to use. Non-radioactive steroids were either obtained from Ikaparm, Israel or Steraloids, Pawling, N.Y. Dextran-coated charcoal was prepared by mixing 10 g of activated charcoal (Sigma) and 1 g of dextran T 70 (Pharmacia) in 100 ml of buffer. Kieselgel G (Type 60) for thin layer chromatography from E. Merck was used. All other reagents were analytical grade from E. Merck.

Preparation of antigen: 17 α hydroxyprogesterone 3-carboxy methoxym was obtained from Steraloids, Pawling, N.Y. and coupled to bovine serum albumin (BSA) by the mixed anhydride technique as described by Erlanger et al.

- short gestation and gestation complicated by hypertension with special reference to the small-for-dates syndrome *Acta Paediatr Scand* 59 13 1970
- 20 Lindblad H S & Baldesten A The normal venous plasma free amino acid levels of non pregnant women and of mother and child during delivery *Acta Paediatr Scand* 56 37 1967
 - 21 Lindblad H S & Baldesten A Time studies on free amino acid levels of venous plasma during the neonatal period *Acta Paediatr Scand* 58 252 1969
 - 22 Lytle I D, Messing R H, Fisher I & Phebus L Effects of long term corn consumption on brain serotonin and the response to electric shock *Science* 190 692 1975
 - 23 Madras B K, Cohen I I, Messing H, Munro H N & Wurtman R J Relevance of free tryptophan in serum to tissue tryptophan concentrations *Metabolism* 23 1107 1974
 - 24 McCaman M W & Robins F Fluorometric method for the determination of phenylalanine in serum *J Lab Clin Med* 59 885 1962
 - 25 McMenamy R H & Oncley J L The specific binding of L tryptophan to serum albumin *J Biol Chem* 243 1436 1968
 - 26 Milner H D G & Richards B An analysis of birth weight by gestational age of infants born in England and Wales 1967-1971 *J Obstet Gynaecol Br Commonw* 81 956 1974
 - 27 Persson B & Gentz J The pattern of blood lipids, glycerol and ketone bodies during the neonatal period, infancy and childhood *Acta Paediatr Scand* 55 353 1966
 - 28 Persson B & Tunnell H Influence of environmental temperature and acidosis on lipid mobilisation in the human infant during the first two hours after birth *Acta Paediatr Scand* 60 385 1971
 - 29 Perez Cruet J, Chase T N & Murphy D L Dietary regulation of brain tryptophan metabolism by plasma ratio of free tryptophan and neutral amino acids in humans *Nature* 248 693 1974
 - 30 Phillipson O T & Bird E D Plasma glucose, non esterified fatty acids and amino acids in Hungarians *Chorei Clin Sci Molec Med* 53 311 1977
 - 31 Sabitz U, Wolf H & Lausmann S The role of free fatty acids, glycerol, ketone bodies and glucose over the energy metabolism in the mother and fetus during delivery *Biol Neonate* 13 7 1968
 - 32 Stein G, Milton F, Bebbington P, Wood K & Coppen A Relationship between mood disturbance and free and total plasma tryptophan in postpartum women *Br Med J* 11 457 1977
 - 33 Tricklebank M D, Pickard F J & Debbin J Tryptophan and 5-hydroxytryptamine metabolism in the immature rat brain during recovery from a physical *J Neurochem* 28 641 1977
 - 34 Tricklebank M D, Smart J L, Bloxam D L & Curzon G Effects of chronic experimental liver dysfunction and L tryptophan on behaviour *Pharmacol Biochem Behav* (in press)
 - 35 Van Duyn C M & Havel R J Plasma unesterified fatty acid concentration in fetal and neonatal life *Proc Soc Exp Biol Med* 107 599 1969
 - 36 Walkes T P & Udenfriend S A fluorometric method for the estimation of tyrosine in plasma and tissues *J Lab Clin Med* 50 733 1957
 - 37 Young M & Prenton M A Maternal and fetal plasma amino acid concentrations during gestation and in retarded fetal growth *J Obstet Gynaecol Br Commun* 76 333 1969
 - 38 Ziegler F & Berger H Tryptophan metabolism in newborn low birthweight infants *Clin Chim Acta* 78 369 1970

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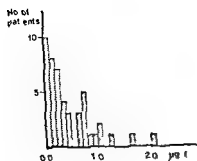


Fig 1 Serum 17 α hydroxyprogesterone levels in 47 children

(<7.6 nmol/l)—found in 62 men and 60 women. The distribution in women is skewed owing to the midcycle and luteal phase peak.

Normal values for children The values observed in 47 children are shown in Fig 1. The chosen reference interval is <1.1 μ g/l (<3.3 nmol/l)—in the first 6 months values up to 2.0 μ g/l (6 nmol/l) have been seen.

Values in CAH

The results of the 6 patients mentioned here are shown in Table 2. Details and comments are given here.

1 At birth this girl was taken to be a boy with hypospadias and testicular retention. So-

dium crisis at age 20 days (serum sodium 119 mEq/l). At the age of one month the condition was thought to be inadequately suppressed so the cortisone dose was raised from 20 to 30 mg daily. As shown this fully suppressed the pathological values.

2 Pseudohermaphroditism at birth and salt loss. Difficult to treat, did not take the cortisone properly, had long lasting menostasia and was adipose. The values are suppressed towards normal values during raised cortisone dose and proper intake.

3 This boy received increased cortisone treatment with the effect shown. He had also extremely large diurnal variations: 11.30 a.m. 112, 11.30 p.m. 3.6 and 8.00 a.m. 161 μ g/l.

4 Pseudohermaphroditism without salt loss. Unmanageable for some time with menostasia and hirsutism, took 25 mg cortisone daily. An increase to 50 mg daily did not change the values much, but almost complete suppression was achieved with 100 mg daily.

5 This girl had an older sister with salt loss CAH, but she had developed quite normally and had never shown any signs of CAH before the age of 5½, when she developed pubic hair. The urinary excretion of 17 KS and pregnanetriol was increased and increased.

Table 2 Values for 17 OH P in patients with CAH

No	Sex	Age studied	Treatment cortisone (mg/24 h)	17 KS (mg/4 h)	Preg nanetriol (mg/24 h)	17 OH P (μ g/l)
1	f	1/12 y	(a) 20 (b) 30	2.3 0.1	0.6 0	184 19
2	f	1½ y	(a) 1.5 ∇ (b) 137.5 ∇ (c) 150 ∇	6.8 6.6 5.5	15.4 7.2 7.5	99 47 171
3	m	8 y	(a) 25 (b) 30	2 2.0	4.9 0.6	65 13
4	f	7½ y	(a) 25 (b) 100	31 3.0	66 0.6	119 22
5	f	5½ y	(a) 0 (b) 0+ACTH (c) 15	2 8.6 1.7	4.3 5.9 0.6	85 99 13
6	f	7 y	(a) 0 (b) 0+ACTH	1.8 7.9	1.2 1.5	2.0 0.8

(8) The reaction mixture was dialyzed three times for 24 hrs against 100 volumes of 0.15 M sodium chloride. By U V spectroscopy the number of steroid residues per mole of BSA was determined to be 11.

Preparation of antiserum The antigen (4 mg/ml in 0.15 M sodium chloride) was mixed with an equal volume of complete Freund adjuvant. Each of six rabbits received subcutaneously 0.1 ml of the suspension once every two weeks for two months. Boosters were then given monthly and blood was drawn 10–12 days after each booster injection.

Assay procedure Serum was equilibrated with a small amount of tritium labelled 17 OH P, denaturated with methanol and extracted with chloroform. After evaporation under nitrogen the extract was purified by thin layer chromatography on silica gel plates with π hexane/ethyl acetate/1,2-dichloroethane/water 100/80/10/1 (vol) as mobile phase. After chromatography, elution from the gel and evaporation of solvent, the extracts were dissolved in phosphate buffer. An aliquot was counted to measure procedural loss while 17 OH P was quantitated in the remaining part by radioimmunoassay with charcoal separation. All results were corrected individually for procedural losses.

Evaluation

Specificity The most suitable antiserum was chosen according to the equilibrium constant for the antigen antibody reaction and the cross reactions with other steroids. The equilibrium constant K was 5×10^{10} l/mol calculated from a Scatchard plot.

Measured cross reactions were as shown in Table 1. It will be seen that the accumulated interference was about 20% at physiological concentrations, mainly owing to progesterone and cortisol. Analysis of 15 normal sera with and without chromatography confirmed this; the values were (mean) 23% higher without chromatography. In a patient with Cushing's syndrome the value without chromatography was 200% higher. As progesterone rises considerably in women in the luteal phase we found it necessary to add a chromatographic purification step. By thin layer chromatography in the system used 17 OH P was separated from the other steroids mentioned in Table 1. It is to be noted that R_f for cortisol, 17 OH P and progesterone is 0.04, 0.39 and 0.61 respectively.

Recovery of 17 OH P added to serum was found to be $104 \pm 6.5\%$ (S.D.) ($n=22$) corrected for procedural losses. The total loss during procedure was $50 \pm 10\%$ (S.D.). Six sera with raised 17 OH P concentration were analyzed at full, half and fifth volume—results were not dependent on the serum volume used.

Reproducibility was studied by analyzing five pools in 12–14 series. The mean coefficient of variation was 6% within the series (S.A. minimum 0.1 μ g/l) and 7% between the series (S.A. minimum 0.2 μ g/l). S.A.=analytical standard deviation.

Sensitivity The detection limit defined as two analytical standard deviations is 0.4 μ g/l.

Other methods

Total 17 AS in urine Callow's modification of the Zimmerman reaction (Statens Seruminstitut). Normal values

Table 1 Cross reactions with different steroids

Steroid	Cross reaction	Relative interference
17 α OH Progesterone	=100	=100
Progesterone	70	10 ^a
17 α OH Pregnenolone	3	3
Pregnenolone	0.2	<1
Cortisol	0.02	<6
11 Deoxycortisol	0.6	<3
Corticosterone	0.02	<1
11 Deoxycorticosterone	0.7	<1
Testosterone	0.07	<0.07
5 α Dihydrotestosterone	0.02	<0.01
Androstenedione	0.07	<0.01
Estradiol 17 β	0.002	<0.01

^a Relative interference signifies cross reaction corrected for the relative physiological serum concentrations.

^b In women in the luteal phase up to 400%.

depend on age: the maximum values are given for the different age groups: 0 year 1 mg/24 hrs—5 years 7 mg/24 hrs—10 years 4 mg/24 hrs—15 years 8 mg/24 hrs—20 years 16 mg/24 hrs (women).

Pregnanetriol in urine Gaschromatography after converting to etiocholanolone (Medi Lab). Normal values in children below 1 mg/24 hrs.

MATERIAL

Normal children

From children who had a venipuncture done during the study of their disease 2 ml of serum was taken for analysis. None of the children had clinical or anamnestic signs of hormonal disease (but may have epilepsy, asthma, different infections). The sixteen girls and thirty-one boys were distributed according to age: 8 days to 1 year 13, 1 to 6 years 17, 6 to 15 years 17. The greater part of the blood sampling was done between 8 a.m. and 10 a.m.—a few samples were taken between 11 a.m. and 3 p.m. in the out patient department.

Patients with CAH

The diagnosis was made in the neonatal period in girls with virilized external genitalia (with or without salt loss) and in boys with salt loss—or later when precocious pseudopuberty occurred. The diagnosis rests at increased 17 AS and pregnanetriol excretion which are suppressible with cortisone treatment. In some cases a thorough metabolic pattern excretion was available. All the patients were suffering from 21 hydroxylase deficiency.

RESULTS

Reference values

Reference values for adults (interval between 2.5% and 97.5% fractiles) were <2.5 μ g/l.

matography the authors conclude that chromatography must be used in all 17 OH P analysis

In the present work it was found necessary to add chromatography and thin layer chromatography was found very convenient for routine use

Normal values

The reference values in the papers published vary with the method being highest in the methods without purification. The published reference values for adults correspond well with those published here. During pregnancy there is an increase to 10–40 $\mu\text{g/l}$ around the 4th week followed by a decrease to below 5 $\mu\text{g/l}$ and a rise during the last weeks (32–33, 37).

Normal values for children are mostly below 3 $\mu\text{g/l}$ varying according to age and to the sensitivity and specificity of the methods. Values are shown in Table 3 and few other papers (1, 27–32).

17 OH P in CAH

Strott et al. (31) determined the secretion rate of 17 OH P to be 113 and 55 mg/24 hrs in a five year-old and a sixteen year old patient with CAH—normal values in adults 1.1–1.9 mg/24 hrs. Weinheimer et al. (34) found values between 130 and 460 $\mu\text{g/l}$ in adrenal venous blood in 10 patients with CAH (14–40 years)—values <100 $\mu\text{g/l}$ in normal controls.

As shown in Table 3 the values of 17 OH P in peripheral blood in untreated patients are found to be between 12 and 860 $\mu\text{g/l}$. The values rise after ACTH stimulation two to five times or more and are suppressed by decadron or cortisone treatment. These findings are in accordance with our experience as illustrated by the cases recorded here.

In untreated patients 17 OH P will thus often be up to several hundred times the value in controls. In extreme cases pregnanetriol may increase to 25 times the upper normal value and 17 KS up to 15 times. This clinical sensitivity is valuable for the diagnosis of

CAH (21 hydroxylase deficiency—and the differential diagnosis between this and other defects (19)). It is easier and faster to take a blood sample than to collect a 24 hour urine—especially in children in a poor condition or with adrenal insufficiency which should be treated immediately. The value of 17 OH P in the differential diagnosis in precocious puberty and premature pubarche (5) is highest when it is studied during stimulation and if necessary steroid suppression.

The usefulness of 17 OH P in the therapeutic monitoring of patients with CAH—if it is easier to make the fine adjustment with 17 OH P than it was with 17 KS and pregnanetriol—is being studied at present. The value of cortisol and ACTH estimation is also being studied.

The extent of the diurnal variation is essential for the timing of the blood sampling. 17 OH P seems to vary like ACTH and cortisol. In normal adults episodic secretion (0–3 $\mu\text{g/l}$) has been demonstrated (35) and integrated curves show a 24 hour variation of a similar size (0.8–1.7 $\mu\text{g/l}$) (13). Strott et al. (31) found that the value at 8 p.m. was about 40% of the value at 8 a.m. (1.0 $\mu\text{g/l}$) and assumed that there would be no variation in patients with CAH. In untreated patients and patients in poor control the highest values are found between 5 a.m. and 11 a.m. (>100 $\mu\text{g/l}$) with a fall during the day (to 40–90 $\mu\text{g/l}$ at 6 p.m.) and a minimum at midnight (about 20 $\mu\text{g/l}$) followed by a new rise (3, 16, 20, 21, 36, 38). In well treated patients there is no or a very small diurnal variation (16–20).

The relatively small diurnal variations found in normal adults and the large difference between normal values and the values in untreated patients whatever the time of day the sampling has been done indicates that diurnal variation does not injure the possibilities of diagnosis. However the sampling time and especially the time which has passed since the last dose of cortisone has been taken may affect the value of 17 OH P in the therapeutic monitoring.

Table 3 Values for 17 OH P in untreated patients with CAH and reference values for children

Ref no	Method	No	Age	Range ($\mu\text{g/l}$)	Reference values children ($\mu\text{g/l}$)
30+31	CPB +	5	5-16 y	40-220	
17	CPB ?	6	4 d -26 y	24-110	0.16-0.59 (5-11 y)
26	CPB +	5	7-18 y	146-222	
7	CPB O	7	6 d -5 y	46-194	<5
9	CPB O	7	<1 month	44% (mean)	
			>1 month	42% (mean)	
23	CPB ?	23	10 d -21 y	40->400	≤ 20
4	CPB O	9	6-12 d	34-250	<10
10	RIA +	12	1 d -40 y	90-578	
25	RIA O	3		200-860	<3
21	CPB +	5	13 d -4 y	12-380	0.39 +/- 0.22
20	RIA +	5	16 d -6 y	60-312	2.1 (<6 weeks)
					0.3 (>6 weeks)
5	RIA +	2	6-7 y	2.56-13.57	0.01-1.37
19	RIA +	8	3 d -10 y	12-700	0.10-0.53
38	RIA +	5	3 d -3 y	180-612	1-6.3
12	CPB ?	7	2 d -3 y	90-380	<0.4
14	RIA (+)	4	?	37-365	0.17 +/- 0.23
16	RIA +	13	21 d -16 y	20-800	0.05-3.15
24	CPB ?	10	7 d -5 y	40->400	
6	RIA +	5	9-19 y		

O signifies without chromatography or other purification + signifies with chromatography or other purification

further during ACTH stimulation to fall again during cortisone treatment. The 17 OH P increased more than tenfold after ACTH. When treatment with cortisone was continued suppression was complete. The diagnosis was CAH with a late debut.

6 For 8 months this girl had developed pubic hair. She was studied in the same way as patient no. 5 and from the data shown and other studies she was shown to have a premature pubarche. This patient is chosen for comparison and does not have CAH.

DISCUSSION

Methods

Competitive protein binding technique (CPB) was first used in the determination of 17 OH P by Strott & Lipsett 1968 (29). Later other methods were published (7, 9, 15, 21, 23, 27, 28). With the CPB technique preliminary purification is essential (although not always used) because of considerable interference from other steroids, chiefly cortisol and progesterone. In the examination of patients on corti-

sone treatment it is essential to avoid a determination of cortisol.

Radioimmunoassays (RIA) which are more specific and sensitive have been predominant in recent years. The necessity for purification in RIA depends on the specificity of the antibody but methods involving such purification must be considered the most precise. Celite chromatography (2), paper chromatography (35) and sephadex (6, 10, 16, 18, 39) have been used. Hariri et al. (14) used immunological purification with excess of antiserum before carrying out RIA.

Until recently none of the published RIA methods for 17 OH P have shown the specificity of a direct analysis by comparing results with and without chromatographic purification. Forest (11) used a highly specific antiserum in a method including celite chromatography and found comparable results with and without chromatography. Values in pathological states are not given. Tea et al. (32) used a special technique by adding ^3H 17 OH P serving both as tracer for losses and as competitive steroid in immunological reaction. After studying the consequences of omitting chro-

mography the authors conclude that chromatography must be used in all 17 OH P analysis

In the present work it was found necessary to add chromatography and thin layer chromatography was found very convenient for routine use

Normal values

The reference values in the papers published vary with the method being highest in the methods without purification. The published reference values for adults correspond well with those published here. During pregnancy there is an increase to 10–40 $\mu\text{g/l}$ around the 4th week followed by a decrease to below 5 $\mu\text{g/l}$ and a rise during the last weeks (32–33, 37).

Normal values for children are mostly below 3 $\mu\text{g/l}$ varying according to age and to the sensitivity and specificity of the methods. Values are shown in Table 3 and few other papers (1, 27–32).

17 OH P in CAH

Strott et al. (31) determined the secretion rate of 17 OH P to be 113 and 55 $\text{mg}/24 \text{ hrs}$ in a five year-old and a sixteen year old patient with CAH—normal values in adults 1–1.9 $\text{mg}/24 \text{ hrs}$. Weinheimer et al. (34) found values between 130 and 460 $\mu\text{g/l}$ in adrenal venous blood in 10 patients with CAH (14–40 years)—values <100 $\mu\text{g/l}$ in normal controls.

As shown in Table 3 the values of 17 OH P in peripheral blood in untreated patients are found to be between 12 and 860 $\mu\text{g/l}$. The values rise after ACTH stimulation two to five times or more and are suppressed by decadron or cortisone treatment. These findings are in accordance with our experience as illustrated by the cases recorded here.

In untreated patients 17 OH P will thus often be up to several hundred times the value in controls. In extreme cases pregnanetriol may increase to 25 times the upper normal value and 17 KS up to 15 times. This clinical sensitivity is valuable for the diagnosis of

CAH (21 hydroxylase deficiency—and the differential diagnosis between this and other defects (19)). It is easier and faster to take a blood sample than to collect a 24 hour urine—especially in children in a poor condition or with adrenal insufficiency which should be treated immediately. The value of 17 OH P in the differential diagnosis in precocious puberty and premature pubarche (5) is highest when it is studied during stimulation and if necessary steroid suppression.

The usefulness of 17 OH P in the therapeutic monitoring of patients with CAH—if it is easier to make the fine adjustment with 17 OH P than it was with 17 KS and pregnanetriol—is being studied at present. The value of cortisol and ACTH estimation is also being studied.

The extent of the diurnal variation is essential for the timing of the blood sampling. 17 OH P seems to vary like ACTH and cortisol. In normal adults episodic secretion (0–3 $\mu\text{g/l}$) has been demonstrated (35) and integrated curves show a 24 hour variation of a similar size (0.8–1.7 $\mu\text{g/l}$) (13). Strott et al. (31) found that the value at 8 p.m. was about 40% of the value at 8 a.m. (1.0 $\mu\text{g/l}$) and assumed that there would be no variation in patients with CAH. In untreated patients and patients in poor control the highest values are found between 5 a.m. and 11 a.m. (>100 $\mu\text{g/l}$) with a fall during the day (to 40–90 $\mu\text{g/l}$ at 6 p.m.) and a minimum at midnight (about 20 $\mu\text{g/l}$) followed by a new rise (3, 16, 20, 21, 36, 38). In well treated patients there is no or a very small diurnal variation (16, 20).

The relatively small diurnal variations found in normal adults and the large difference between normal values and the values in untreated patients whatever the time of day the sampling has been done indicates that diurnal variation does not injure the possibilities of diagnosis. However the sampling time and especially the time which has passed since the last dose of cortisone has been taken may affect the value of 17 OH P in the therapeutic monitoring.

Table 3 Values for 17 OH P in untreated patients with CAH and reference values for children

Ref no	Method	No	Age	Range ($\mu\text{g/l}$)	Reference values children ($\mu\text{g/l}$)
30+31	CPB +	5	5-16 y	40-220	0.16-0.59 (5-11 y)
17	CPB *	6	4 d - 26 y	24-330	
26	CIB +	5	7-18 y	146-222	
7	CPB O	7	6 d - 5 y	46-194	
9	CIB O	7	<1 month	448 (mean)	<5
			<1 month	423 (mean)	
23	CPB *	23	10 d - 23 y	40-2400	<20
4	CPB O	9	6-12 d	34-250	<10
10	RIA +	12	3 d - 40 y	90-528	<3
25	RIA O	3		200-860	
21	CIB +	5	13 d - 4 y	12-380	0.39 +/- 0.22
20	RIA +	5	16 d - 6 y	60-312	2.1 (<6 weeks)
					0.3 (>6 weeks)
5	RIA +	2	6-7 y	246-1357	0.03-1.37
19	RIA +	8	3 d - 10 y	12-700	0.10-0.51
38	RIA +	6	3 d - 3 y	180-612	1-6.3
12	CPB *	7	2 d - 3 y	90-380	<0.4
14	RIA (+)	4	?	37-365	0.17 +/- 0.23
16	RIA +	13	21 d - 16 y	20-800	0.05-1.15
24	CPB *	10	7 d - 5 y	40-2400	
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- durch Radioimmunologischen Nachweis von 17 α Hydroxyprogesteron *Monatsschr Kinderheilkd* 127 6 1974
- 6 Simopoulos A P Marshall J R Delea C S & Bartter F C Studies on the deficiency of 17 hydroxylation in patients with congenital adrenal hyperplasia *J Clin Endocrinol* 37 438 1971
 - 7 Stewart Bentley M & Horton R 17 α hydroxy progesterone in human plasma *J Clin Endocrinol* 33 547 1971
 - 8 Stone M Khanna K M Nakamura R N Mishell D R & Thorneycroft I H A technique for the assay of 17 hydroxyprogesterone in serum using celite column chromatography and competitive protein binding *Steroids* 18 161 1971
 - 9 Strott C A & Lipsett M B Measurement of 17 hydroxyprogesterone in human plasma *J Clin Endocrinol* 28 1476 1968
 - 10 Strott C A Yoshimura T Bardin C W & Lipsett M B Blood progesterone and 17 hydroxyprogesterone levels and production rates in a boy with virilizing congenital adrenal hyperplasia *J Clin Endocrinol* 28 1085 1968
 - 11 Strott C A Yoshimura T & Lipsett M B Plasma progesterone and 17 hydroxyprogesterone in normal men and children with congenital adrenal hyperplasia *J Clin Invest* 48 930 1969
 - 12 Tea N T Castanier M Roger M & Scholler R Simultaneous radioimmunoassay of plasma progesterone and 17 hydroxyprogesterone: normal values in children in men and in women throughout the menstrual cycle and in early pregnancy *J Steroid Biochem* 6 1509 1975
 - 13 Tulshinsky D & Summer H H Sources of plasma 17 α hydroxyprogesterone in human pregnancy *J Clin Endocrinol Metab* 35 799 1972
 - 14 Weinheimer B Oertel G W Leppla W Blaise II & Bette L Adrenal venous plasma concentration of 17 OH progesterone and urinary pregnanetriol excretion in 10 cases of adrenogenital syndrome (abstract) *Acta Endocrinol (Kbh) Suppl* 100 67 1965
 - 15 West C D Mahajan B K Chavre V J Nabors C J & Tyler F H Simultaneous measurement of multiple plasma steroids by radioimmunoassay demonstrating episodic secretion *J Clin Endocrinol Metab* 36 1230 1973
 - 16 West C D Stanchfield J M Atcheson J B Rallison M L & Tyler F H Circadian variation in plasma ACTH and cortisol and its precursors in congenital adrenal hyperplasia (abstract) *Clin Res* 21 190 1973
 - 17 Yoshimura T Strott C A Marshall J R & Lipsett M B Corpus luteum function in early pregnancy *J Clin Endocrinol* 29 725 1969
 - 18 Youssefnejadian E & David M Early diagnosis of congenital adrenal hyperplasia by measurement of 17 hydroxyprogesterone *Clin Endocrinol* 4 451 1975
 - 19 Youssefnejadian E Florensa M Collins W P & Sommerville I F Radioimmunoassay of 17 hydroxyprogesterone *Steroids* 30 773 1977

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We may conclude from the literature and the data here shown that the measurement of 17 OH P by a specific method such as that described has great importance for the diagnosis and perhaps also for the therapeutic monitoring of CAH (21 hydroxylase deficiency). Possibly it may also be used in the differential diagnosis in Cushing's syndrome. During pregnancy there is a rather large variation in 17 OH P but it has been suggested (22) to use the analysis to show the presence of chorionic cysts in patients with gestational trophoblastic neoplasms.

REFERENCES

- 1 Abraham G F, Hopper A, Tulchinsky D, Swerdloff R S & Odell W D. Simultaneous measurement of plasma progesterone, 17 hydroxyprogesterone & estradiol 17B by radioimmunoassay. *Anal Letters* 4 325 1971
- 2 Abraham G E, Swerdloff R S, Tulchinsky D, Hopper A & Odell W D. Radioimmunoassay of plasma 17 hydroxyprogesterone. *J Clin Endocrinol* 33 42 1971
- 3 Atherden S, Barnes N D & Grant D B. Circadian variation in plasma 17 hydroxyprogesterone in patients with congenital adrenal hyperplasia. *Arch Dis Child* 47 602 1972
- 4 Atherden S M, Edmunds A T & Grant D B. Plasma 17 hydroxyprogesterone in newborn infants with congenital adrenal hyperplasia and in infants with normal adrenal function. *Arch Dis Child* 49 192 1974
- 5 August G P, Hung W & Myles D M. Plasma androgens in premature pubarche. Value of 17 hydroxyprogesterone in differentiation from congenital adrenal hyperplasia. *J Pediatr* 87 246 1975
- 6 Bacon E, Spencer M L & Kelch R P. Effect of cortisol treatment on hormonal relationships in congenital adrenal hyperplasia. *Clin Endocrinol* 6 113 1977
- 7 Barnes N D & Atherden S M. Diagnosis of congenital adrenal hyperplasia by measurement of plasma 17 hydroxyprogesterone. *Arch Dis Child* 47 62 1972
- 8 Erlanger B F, Borek P, Bever S M & Lieberman E. *J Biol Chem* 228 713 1958
- 9 Franks R C. Screening tests for congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 35 831 1972
- 10 Franks R C. Plasma 17 hydroxyprogesterone, 21 deoxycortisol and cortisol in congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 39 1099 1974
- 11 Forest M G. Use of highly specific antibodies against 17 OH progesterone in a simplified non chromatographic RIA and in the simultaneous determination of four sex hormones in human plasma. *Horm Res* 7 260 1976
- 12 Gillet P. Interet des dosages plasmatiques d'active renine, testostérone, 17 alpha hydroxyprogesterone et ACTH dans le diagnostic et la surveillance de hyperplasies congénitales des surrenales par déficit en 21 hydroxylase. Lyon 1975. Thesis
- 13 Gutai J P, Meyer W J, Kowarski A A & Migeon C J. Twenty four hour integrated concentrations of progesterone, 17 hydroxyprogesterone and cortisol in normal male subjects. *J Clin Endocrinol Metab* 44 116 1977
- 14 Hami M, Rosler A & Rabinowitz D. A non chromatographic radioimmunoassay for 17 alpha hydroxyprogesterone. *J Clin Endocrinol Metab* 40 863 1975
- 15 Holmdahl T H & Johansson B B. Peripheral plasma levels of 17 alpha hydroxyprogesterone, progesterone and oestradiol during normal menstrual cycle in women. *Acta Endocrinol (Kbh)* 71 743 1977
- 16 Hughes I A & Winter J S D. The application of serum 17 OH progesterone radioimmunoassay to the diagnosis and management of congenital adrenal hyperplasia. *J Pediatr* 85 766 1976
- 17 Jenner M E, Grumbach M M & Kaplan S L. Plasma 17 OH progesterone in maternal and umbilical cord plasma in children and in congenital adrenal hyperplasia (abstract). *Pediatr Res* 4 340 1970
- 18 Janne O, Apter D & Vihko R. Assay to test progesterone, progesterone and 17 alpha hydroxyprogesterone in human plasma by radioimmunoassay after separation in hydroxyalkoxypropyl sephadex. *J Steroid Biochem* 5 155 1974
- 19 Janne O, Perheentupa J, Viimikka L & Vihko R. Plasma pregnenolone, progesterone, 17 hydroxyprogesterone, testosterone and 5-alpha-dihydrotestosterone in different types of congenital adrenal hyperplasia. *Clin Endocrinol* 4 39 1975
- 20 Lippe B M, LaFranchi S H, Lavin N, Parlow A, Coyotupa J & Kaplan S A. Serum 17 alpha hydroxyprogesterone, progesterone, estradiol and testosterone in the diagnosis and management of congenital adrenal hyperplasia. *J Pediatr* 85 187 1974
- 21 Loras B, Roux H, Audi Parera L, David M & Bertrand J. 17-alpha hydroxyprogesterone plasmatique dans l'hyperplasie congénitale des surrenales par déficit en 21 hydroxylase traitée et non traitée. *Biomedicine* 21 317 1974
- 22 Osathanondh B, Goldstein D P, Tulchinsky D & Finn A E. Serum 17 alpha hydroxyprogesterone in patients with gestational trophoblastic neoplasms. *Obstet Gynaecol* 49 77 1977
- 23 Pham Huu Trung M T, Gourmelen M & Girard F. The simultaneous assay of cortisol and 17 alpha hydroxyprogesterone in the plasma of patients with congenital adrenal hyperplasia. *Acta Endocrinol (Kbh)* 74 316 1973
- 24 Pham Huu Trung M T, Raux M C, Gourmelen M, Baron M C & Girard F. Plasma aldosterone concentrations related to 17 alpha hydroxyprogesterone in congenital adrenal hyperplasia. *Acta Endocrinol (Kbh)* 87 572 1976
- 25 Schnakenburg K V, Bidlingmaier F & Knorr D. Diagnostik und Verlaufskontrolle des adrenogenitalen Syndroms vom Typ des 21 Hydroxylase-defektes.

INCREASE IN BILIRUBIN BINDING TO ALBUMIN WITH CORRECTION OF NEONATAL ACIDOSIS

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ABSTRACT Kozuki K, Oh W, Widness J and Cashore W (Department of Pediatrics Women and Infants Hospital Providence Rhode Island USA) Increase in bilirubin binding to albumin with correction of neonatal acidosis. *Acta Paediatr Scand* 68 213 1979. Twenty six serial measurements of free bilirubin concentration and apparent association constant of bilirubin for albumin (K_a) at a bilirubin albumin molar ratio of 0.3 were performed and compared with baseline values in 11 newborn infants with acidosis before treatment and during recovery from acidosis. When arterial pH was corrected from 7.12 ± 0.02 (Mean \pm S.E.M.) to 7.34 ± 0.02 there was a significant decrease in serum free bilirubin concentration and a significant increase in the K_a at molar ratio 0.8. The data offer *in vivo* evidence that correction of acidosis in the neonate results in an improvement of the apparent bilirubin binding affinity of albumin.

KEY WORDS Newborn bilirubin binding albumin acidosis

Neonatal acidosis is associated with an increased risk of bilirubin encephalopathy (kernicterus). Stern et al (15) reported an increased incidence of kernicterus in small sick premature infants following acidosis and hypothermia. Gartner et al (6) and Keenan et al (9) have reported similar findings in sick preterm infants. Animal studies have shown increased bilirubin uptake in the brains of guinea pigs subjected to respiratory acidosis (4) and of Rhesus monkeys asphyxiated at birth (10). *In vitro* Odell and associates have observed a shift in the spectral curve of bilirubin from the bound toward the unbound state at lowered pH (13). Nelson et al however using a cell culture model reported that acidosis affects the distribution and toxicity of bilirubin by increasing the deposition of bilirubin in cells rather than by decreasing the affinity of albumin for bilirubin (12). In the current study we have measured bilirubin-albumin association constants in 11 newborn infants during and after recovery from acidosis to evaluate the effect of acidosis on bilirubin binding to albumin in neonatal sera.

MATERIAL AND METHODS

The subjects were 11 newborn infants admitted to the Newborn Special Care Unit of the Women and Infants Hospital of Rhode Island with respiratory distress and acidosis. The primary diagnoses included respiratory distress syndrome in 6 and birth asphyxia in 5 infants. Complications included pneumothorax in 2 infants, aspiration pneumonia in 2, seizures in 2 and hemorrhagic shock in one infant. Nine of the 11 infants survived. The average birth weight was 3800 g (range 1530-5775) and the average gestational age was 36.6 weeks (range 31-42). The criterion for admission to the study was an initial arterial blood pH of less than 7.25. At the time of the first and subsequent arterial pH determinations (the 11 infants had a total of 6 follow up measurements) 0.5 ml of unheparinized blood was drawn from the arterial catheter for the bilirubin binding studies. Specimens were obtained at the time of each pH determination until the acidosis was corrected to a pH value above 7.30.

For the correction of acidosis the patients received sodium bicarbonate according to the formula

Base deficit (mEq/l) \times body wt (kg) \times 0.3 = mEq sodium bicarbonate given

The 0.9 M bicarbonate was diluted 1:1 with sterile water and was given over 30-60 minutes. This infusion of bicarbonate was repeated once if the metabolic acidosis persisted. In addition positive pressure assisted ventilation was required in 5 infants who had combined respiratory and metabolic acidosis (2), apneic spells (1) or persistent hypoxemia despite application of continuous positive airway pressure (7).

free bilirubin nmol/l)	
B/A=0.8 pH 7.4	
T=34°C	$Ka \times 10^3 M$
1.5	0.95 ± 0.08
1.4	1.29 ± 0.10
0.005	<0.01

an increase in apparent bilirubin albumin association constant (K_a) from $11.95 \pm 0.08 \times 10^3 M^{-1}$ to $1.29 \pm 0.1 \times 10^3 M^{-1}$ ($p < 0.01$). Rectal temperature averaged $98.1 \pm 0.02^\circ F$ at the time the studies were begun and remained stable throughout the period of study. Serum glucose was 54 ± 7 mg/100 ml at the beginning of the study, one value was 24 mg/100 ml and the remaining patients had serum glucose values in excess of 37 mg/100 ml.

As shown in Fig. 1 increases in blood pH were associated with significant decreases ($r = 0.640$, $p < 0.005$) in free bilirubin (i.e. after dilution of serum and addition of bilirubin to a B/A ratio of 0.8) and significant increases in apparent K_a ($r = 0.627$, $P < 0.005$). Reduction in base deficit was associated with a significant decrease in free bilirubin and significant increase in K_a ($r = 0.473$, $p < 0.02$ for both correlations (graphs not shown)). There was no correlation between changes in free bilirubin or K_a and changes in postnatal age, time after entry into the study, P_a or P_e . Acidosis recurred in two patients after early correction and in both this recurrence was accompanied by a decrease in K_a .

DISCUSSION

Acidosis was associated with a reduction in apparent bilirubin binding affinity even when the free bilirubin assays were performed at pH 7.4. Using Sephadex gel filtration at a constant pH of 7.4 we have previously shown that the

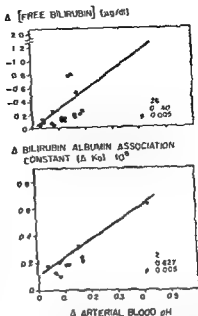


Fig. 1 Decrease in free bilirubin concentration (upper part) and increase in bilirubin-albumin association constant (lower part) with increasing arterial blood pH during correction of neonatal acidosis: serial observations in 11 infants. There are 76 comparisons since multiple repeat determinations were made in 6 patients. Free bilirubin was measured under the following conditions: pH 7.4, $T = 34^\circ C$ and bilirubin-albumin molar ratio = 0.8 after dilution of serum and addition of bilirubin.

bilirubin binding capacity is higher in healthy clinically stable infants than in those with neonatal acidosis and respiratory distress (2, 3). In those studies sequential changes in arterial blood pH were not documented in relation to changes in bilirubin binding characteristics; an association between changes in acid-base balance and changes in bilirubin binding is shown in the current report.

Using the peroxidase method Nelson et al (12) and Jacobsen & Brodersen (7) have found that *in vitro* the bilirubin binding to albumin is not influenced by changes in pH at the range of 7 to 9.3. The chemical structure of bilirubin itself also precludes the possibility that its high affinity binding to the albumin molecule would be affected by hydrogen ion concentration (1).

When Odell et al (13) lowered pH of their reaction medium from 7.4 to 6.8 *in vitro* a shift was observed in the spectral curve of the

Table 1 Acid-base albumin and bilirubin values in 11 newborn infants (26 determinations) before and after correction of acidosis

	Age (hrs)	Arterial blood pH	P _i (mmHg)	P _{co} (mmHg)	Base deficit (mEq/l)	Albumin (g/100 ml)	Indirect Bilirubin (mg/100 ml)
Before correction	9.6±2.3	7.12±0.02	64.4±8.3	34.1±4.7	-17.6±2.2	3.35±0.0	1.9±0.5
After correction	18.5±3.5	7.34±0.02	84.3±11	28.9±3.9	-7.8±1.7	3.05±0.15	2.8±0.1
<i>p</i> *	—	—	<i>n</i> s	<i>n</i> s	<0.001	<i>n</i> s	<0.05

* All values are $M \pm S.E.M.$

* Paired *t* test

* Values for free bilirubin and apparent *K_a* obtained at B/A molar ratio=0.8 pH 7.4 and 24°C after dilution of serum and addition of bilirubin

Serum albumin and total and direct bilirubin concentrations were determined in duplicate by standard methods (5–11) precision of the methods was ± 0.1 g/100 ml at 4 g/100 ml for albumin (coefficient of variation=2.5±1%) and ± 0.3 mg/100 ml at 10 mg/100 ml for bilirubin determinations (coefficient of variation 3±2%). Free bilirubin concentrations after dilution of the serum (25 microliters in 1.0 ml of buffer) and addition of crystalline bilirubin to achieve the desired bilirubin-albumin molar ratio were determined in duplicate by the horseradish peroxidase method (8) with a precision of ± 1 nmol/l at 25 nmol/l (coefficient of variation 4±2%). Aliquots of 25 microliters of serum were used for each free bilirubin determination and the assays were carried out in 1.0 ml of 1/15 M sodium phosphate buffer pH 7.4 at 24°C. To correct for postnatal changes in serum bilirubin and albumin concentration the bilirubin to albumin molar ratio of each serum was raised to 0.8 by addition of crystalline bilirubin (Sigma) in 0.1 NaOH and the free bilirubin assay was performed at this concentration. The apparent association constant *K_a* was calculated from the law of mass action

$$K_a = \frac{(BA)}{(b) \times (a)}$$

in which *K_a*=the apparent association constant of bilirubin for albumin (BA)=the molar concentration of the bilirubin-albumin complex (b)=the molar concentration of free bilirubin and (a)=the molar concentration of albumin without bound bilirubin

At a bilirubin-albumin ratio of 0.8 bilirubin would be bound to 80% (0.8) of its primary binding sites on albumin while 20% (0.2) of the albumin sites would contain no bilirubin giving relative concentrations of 0.8 for bound bilirubin (BA) and 0.2 for albumin without bound bilirubin (a)

The molecular weight of bilirubin was assumed to be 585 of albumin 69000 and the indirect was assumed to equal the bound bilirubin concentration

In 8 samples taken from 4 of the 11 study infants sufficient sample aliquots were obtained to analyze the apparent bilirubin-albumin association constant using both the molar ratio formula above and Scatchard graph analysis in the same specimen the results showed good agreement between the two methods for the determinations of

K_a which averaged $0.85 \pm 0.08 \times 10^4$ M⁻¹ ($M \pm S.E.M.$) by Scatchard plot and $0.93 \pm 0.10 \times 10^4$ M⁻¹ by direct calculation from the formula

Changes in free bilirubin concentrations and association constants were analyzed by paired *t* test. Relationships between changes in free bilirubin or *K_a* and changes in postnatal age pH *P_{co}* or base deficit were determined by regression analysis

RESULTS

A total of 26 serial determinations of arterial blood gas and acid-base values free bilirubin and apparent association constant were made during correction of acidosis in the 11 infants and were compared with baseline values. Pre and post-correction acid-base values and blood gas tensions are shown in Table 1. When first studied at an average age of 9.6 ± 2.3 hours ($M \pm S.E.M.$) the infants had an average arterial blood pH of 7.12 ± 0.02 . Correction of acidosis to pH 7.34 ± 0.02 was achieved by 18.5 ± 3.5 hours of age. Correction of acidosis was accompanied by an increase in *P_O* a slight fall in *P_{co}* and a change of +9.8 mEq/l in the base deficit. From the beginning of the study until the time of pH correction serum albumin concentration did not change significantly (3.35 ± 0.02 to 3.05 ± 0.15 g/100 ml) there was an increase in indirect bilirubin concentration (1.9 ± 0.5 to 2.8 ± 0.2 mg/100 ml). At a bilirubin to albumin molar ratio of 0.8 correction of acidosis was accompanied by a significant decrease in free bilirubin concentration from 42 ± 5 to 31 ± 4 nmol/l ($p < 0.005$) and

Kernicterus in asphyxiated newborn rhesus monkeys
Exp Neurol 9 43 1964

- 11 Martinek R ■ Improved micromethod for determination of serum bilirubin *Clin Chim Acta* 13 161 1966

- 1 Nelson T Jacobsen J & Wennberg R P Effect of pH on the interaction of bilirubin with albumin and tissue culture cells *Pediatr Res* 8 963 1974

- 13 Odell ■ B Cohen S N & Kelly P C Studies in kernicterus II The determination of the saturation of serum albumin with bilirubin *J Pediatr* 74 14 1969

- 14 Odell ■ ■ Cukier J O Ostrea E M Jr et al The influence of fatty acids on the binding of bilirubin to albumin *J Lab Clin Med* 89 95 1977

- 15 Stern L Doray B Chan ■ & Schiff D Bilirubin metabolism and the induction of kernicterus In Bergsma D and Blondheim S H (eds) *Bilirubin metabolism in the newborn (II)* American Elsevier Publ Co Inc New York 1976 pp 55- 61

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bilirubin-albumin complex with a decrease in absorbance at 460 nm this spectral shift was initially interpreted as suggesting dissociation of the complex (13). However in a more recent study (14) the same workers have postulated a shift of bilirubin from primary to secondary binding sites under the influence of strong competitors (e.g. fatty acids) resulting in spectral changes not necessarily related to a release of bilirubin. Other investigators have also interpreted these spectral changes as representing pH induced alterations in the spectrum of the complex rather than increased dissociation of bilirubin from albumin (7, 12). In the present studies all binding assays were performed at the same pH so that the increases in free bilirubin and the decreases in the bilirubin-albumin dissociation constant associated with neonatal acidosis were not effects of hydrogen ion concentration alone. It is of interest that the increment in the binding of bilirubin to albumin with the correction of acidosis was associated with a significant reduction in the base deficit (Table 1). One may speculate that acidosis is associated with the presence of endogenous anions (which may themselves contribute to metabolic acidosis) capable of displacing bilirubin from its albumin binding sites and thereby increasing plasma free bilirubin. With correction of acidosis a concomitant improvement in the circulatory and metabolic status of the infants may reduce the effects of displacers by enhanced clearance, reduction in their formation or changes in the affinity of anions other than bilirubin for albumin binding sites.

To control the reaction rate and molar ratio and to make use of small aliquots of serum free bilirubin concentrations in these studies were determined at 24°C after dilution of the serum and addition of bilirubin the values reported for the apparent association constants were obtained under those conditions. Estimation of free bilirubin and K_a at 37°C in undiluted serum is technically more difficult and would require a much larger serum sample (a limiting factor in the study of small infants) but

if technically feasible would more nearly approximate *in vivo* conditions.

The concentration of free bilirubin which is toxic to the human central nervous system *in vivo* has not been determined. Acidosis appears to be associated with an increase in the free bilirubin concentration furthermore acidosis also enhances the deposition of free bilirubin in the tissues and/or increases the susceptibility of the tissues to bilirubin toxicity as shown *in vitro* in several studies (4, 10, 12, 13). Avoidance of or prompt correction of acidosis in jaundiced newborns appears important to maintain the optimal albumin binding characteristics and to protect the potentially susceptible tissue sites.

REFERENCES

- 1 Brodersen R. Prevention of kernicterus based on recent progress in bilirubin chemistry. *Acta Paediatr Scand* 66: 625, 1977.
- 2 Cashore W J, Horwich A, Karotkin E H & Oh W. The influence of gestational age and clinical status on bilirubin binding capacity in newborn infants determined by Sephadex G 25 gel filtration technique. *Am J Dis Child* 131: 898, 1977.
- 3 Cashore W J, Horwich A & Oh W. Relationship of serum total bilirubin binding capacity to infant maturity and clinical status. *Pediatr Res* 10: 471, 1976 (Abstract).
- 4 Diamond I & Schmid R. Experimental bilirubin encephalopathy. The mode of entry of bilirubin into the central nervous system. *J Clin Invest* 46: 618, 1966.
- 5 Dumas B T, Watson W A & Biggs M G. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 31: 87, 1971.
- 6 Garner L M, Synder R N, Chabon R H & Bernstein J. Kernicterus: high incidence in pretermatures with low serum bilirubin concentrations. *Pediatrics* 45: 906, 1970.
- 7 Jacobsen I & Brodersen R. The effect of pH on albumin-bilirubin binding affinity. In Bergsma D & Blondheim S H (eds.) *Bilirubin metabolism in the newborn (III)*. American Elsevier Publ Co Inc, New York, 1976, pp 175-178.
- 8 Jacobsen J & Wennberg R P. Determination of unbound bilirubin in the serum of newborns. *Clin Chem* 20: 783, 1974.
- 9 Keenan W J, Perlstein P H, Light J J & Sutherland J M. Kernicterus in small sick infants receiving phototherapy. *Pediatrics* 49: 652, 1972.
- 10 Lucey J F, Hubbard E & Behrman R E et al.

- kernicterus in asphyxiated newborn rhesus monkeys
Exp Neurol 9 43 1964
- Martinek R G Improved micromethod for determination of serum bilirubin *Clin Chim Acta* 13 161 1966
- Nelson T Jacobsen J & Wennberg R P Effect of pH on the interaction of bilirubin with albumin and tissue culture cells *Pediatr Res* 8 963 1974
- Odell G H Cohen H N & Kelly P C Studies in kernicterus II The determination of the saturation of serum albumin with bilirubin *J Pediatr* 74 14 1969
- Odell G B Cukier J O Ostrea E M Jr et al The influence of fatty acids on the binding of bilirubin to albumin *J Lab Clin Med* 89 795 1977
- 15 Stern L Doray H Chan G & Schiff D Bilirubin metabolism and the induction of kernicterus In Bergsma D and Blondheim H H (eds) *Bilirubin metabolism in the newborn (II)* American Elsevier Publ Co Inc New York 1976 pp 255-763

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BENIGN PAROXYSMAL TORTICOLLIS IN INFANCY

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ABSTRACT Sanner G and Bergström B (Departments of Paediatrics and Otolaryngology Central Hospital Karlstad Sweden) Benign paroxysmal torticollis in infancy. *Acta Paediatr Scand* 68 219 1979.—Benign paroxysmal torticollis is a condition characterized by attacks of head tilting often accompanied by lateral curvature of the trunk. The first onset of these attacks usually occurs during the first months of life and they recur with a remarkable periodicity for 6–12 months after which time they gradually subside in intensity and frequency. As a rule the attacks have ceased completely before the age of 2 years but in some cases they continue in a modified form as attacks of ataxia. The aetiology is unknown and there is no evidence of bilateral peripheral vestibular disturbances as has been suggested in earlier reports. The ataxia seen in some cases rather suggests a dysfunction of the cerebellum or of the vestibulo-cerebellar connections. Four own patients with this syndrome are presented and discussed.

KEY WORDS Paroxysmal torticollis, infancy, periodic disorders, paroxysmal ataxia.

In 1969 Snyder (14) reported 12 children with a paroxysmal condition appearing in infancy and characterized by periods of torticollis like posture of the head.

A further case was reported by Gourley (8). Chutanan (3) presented 5 children with the same condition and added to the clinical picture the occurrence of lateral curvature of the trunk. Two of the children also showed an abnormal posture on vertical suspension. No further reports of this condition seem to have appeared in the literature and the condition is not to be found in ordinary textbooks of paediatrics or child neurology.

In 1976 the authors presented two children with paroxysmal torticollis at the 14th Scandinavian Pediatric Congress (13). Since then we have recognized two further cases and will present our experiences from 4 children with this peculiar condition.

CASE HISTORIES

Case 1

J N Female. First and only child of healthy parents who were not consanguineous. Her paternal grandfather suffered from a torticollis in early life. Her father had a

torticollis from early infancy to 1.5 years of age and according to his mother it had been of the same type as in J N. Her pre- and neonatal history was normal as well as her psychomotor development.

From the age of one month attacks appeared when the girl kept her head mostly to the left side but also to the right. Her head was also slightly retracted. When lying prone the baby could move her head freely. In other positions straightening of her neck caused irritation. Otherwise the baby seemed to be perfectly well during the attacks. The duration of the attacks were 3–7 days with a maximum of head tilting on the second day after which the condition successively subsided. The attacks were recognized by her parents when the baby woke up in the morning.

During the first 6 months the attacks recurred very regularly with a free interval of 14 days. Later on the frequency diminished as well as the severity of the attacks. Treatment with carbamazepine was tried but had no effect. The last attack was seen at 13 months of age. The girl is now 2.5 years old and is healthy and well developed in all respects.

Examinations. Physical and neurological examinations at several occasions revealed no abnormalities except for the torticollis. An ophthalmological examination was normal as was an electroencephalogram.

Oto-neurological examination including electronystagmography with caloric tests in a free interval revealed no abnormalities. Repeated hearing tests the latest at 2.5 years of age were normal.

Case 2

J II Female. The third of three children of healthy parents who were not consanguineous. A brother of J II



Fig 2 Paroxysmal torticollis in case 2 at 9 months of age showing curvature of the trunk and extension of the right leg (ink drawing from 8 mm movie)

recognized the condition in the morning. At the onset the baby seemed to sweat and was irritable but showed no other signs of distress during the attacks. At examination during an attack the baby was at first seen sleeping supine with her head bent to the left. Awakening she bent her head to the right and retracted it. Slight curvature of the trunk but no change of the tone in her arms or legs was noticed. The position was accentuated when the girl was held upright.

The girl is now 8 months old and except for the regularly recurring attacks healthy and well developed.

Examinations. Physical and neurological examinations were normal. Roentgen studies of the spine, electroencephalography during an attack and echo-encephalography revealed no abnormalities. ENG. Normal caloric reactions.

DISCUSSION

The attacks of head tilting in these four cases evidently represent the same condition as was reported by Snyder (14), Gourley (8) and Chutonian (3). The main clinical characteristics

Table 1 Main clinical features of benign paroxysmal torticollis according to cases reported

Paroxysmal occurrence of head tilting and—retraction
Often lateral curvature of the trunk
Sometimes extension pattern of one leg
Usually more abnormal posture in supine and upright positions
Sometimes distress and abnormal rolling of the eyes at onset of attacks
Regular periodicity of attacks for a long time
Duration of attacks: hours—days
Onset within the first year of life
Recovery from 1 year to 5 years of age
Female preponderance

of the condition according to this series and reports from the literature are shown in Table 1.

In more severe cases (case 2) extension of the extremities on one side are also seen. This involvement of the extremities has not been reported before.

In some cases the onset of an attack is accompanied by distress and vomiting. The first attack usually occurs during the first year of life and we have even seen it during the first week (case 4). The age of recovery is from 1 year to 5 years according to Snyder (14). The duration of attacks varies from 10 min to several days.

The main diagnostic problem is the first attack appearing in a previously healthy infant. Torticollis in infancy may be the first sign of a posterior fossa tumor (6) which therefore must be ruled out especially when accompanied by vomiting.

Recurrent cervical dislocation as well as epilepsy has been discussed (3, 14) but never proved. Electroencephalography also during attacks in 2 of our cases showed no abnormality. However this does not discard epileptic discharge at a lower subcortical level as a cause of paroxysmal torticollis. Therefore we treated 2 of our patients (cases 1 and 2) with carbamazepine which has had excellent effect in spinal seizures (5) as well as in paroxysmal choreo-athetosis (16) but this



Fig. 1 Paroxysmal torticollis in case 2 at 8 months of age (a) in the left side and (b) at the next occasion to the right

has a left-sided sensorineural hearing loss. Several other relatives also have sensorineural hearing loss.

Pre-perinatal and neonatal history as well as psychomotor development was quite normal. From 3 months of age attacks were noted when the girl kept her head to one side (Fig. 1). At the same time her trunk was curved and her contralateral leg rigidly extended with plantar flexion of the foot (Fig. 2). This posture was most pronounced in upright and supine positions but inhibited when prone or crawling.

At the onset of some attacks the mother noticed that the girl's eyes rolled upwards. At one occasion when her neck was straightened rolling of her eyes occurred until her head was tilted again. No engagement of the face and mouth was noted.

The attacks were always recognized in the morning. Especially at the beginning of an attack the girl sometimes seemed unwell, irritated and drowsy.

The duration of the attacks varied from 3–12 hours. The attacks recurred very regularly once a month but from the age of 1 year the frequency gradually diminished. From 1.5 to 2.5 years of age she had about 7 attacks, some very mild but other severe and showing some additional signs. The latest attack was described as follows. She had a balance disturbance and often fell. She walked on a broad base, unsteady with short steps. She had only a slight torticollis and no affection of her trunk or leg. When moving she preferred to crawl with her head bent down. Her favourite position was crouching. An evident tremor of grasping was noticed. Her eyes were seen rolling up at occasions. During the latest attack lasting 12 hours she refused to eat and drink. She vomited twice and a few times she complained of headache. This attack as the others stopped suddenly as a light button is switched off. She then could walk, drink, eat and play as usual. The girl is now 2.5 years old and quite healthy and well developed.

Examinations. Physical and neurological examinations never revealed any abnormalities. Ophthalmological examination, electroencephalography and echocardiography were normal as was electrocardiography

during an attack. Chromatography of the urine showed no abnormal organic acids. Roentgen studies of the skull and cervical spine were normal. Caloric tests during attack and in a free interval showed normal reactions bilaterally registered by ENG. Hearing tests indicate normal hearing.

Case 3

M. J. Female. First and only child. The parents were not consanguineous. Her mother has a slight sensorineural hearing loss on one side. Pre-perinatal and neonatal history was normal. From 2 months of age attacks with the head tilted to the left and sometimes slight curvation of the trunk. During an attack the torticollis gradually appeared within 2 days and faded within 2 weeks. During the attacks the baby was quite well. The attacks occurred very regularly with a free interval of 14 ± 1 days. Gradually the attacks became less visible and were noticed only when she was tired. From 1.5 years of age no attacks were seen. The girl is now 2 years old, healthy and well developed.

Examinations. Physical and neurological examination was normal. Ophthalmological examination and electroencephalography during an attack showed normal results. Roentgen studies of the cervical spine revealed no abnormalities. Rotation test showed normal reactions. (The girl refused to cooperate in caloric tests.) Hearing tests were normal.

Case 4

A. K. II Female. First and only child of healthy parents who were not consanguineous. The prenatal period was normal. Because of a prolonged labour the child was delivered by vacuum extraction. The Apgar score was 8 at 1 min. Two hours after birth two short convulsion episodes were observed. Otherwise the neonatal period was normal.

At 1 week of age a torticollis was noticed which disappeared within 1 week. Thereafter especially right-sided torticollis attacks reappeared regularly with a free interval of 16–19 days and a duration of 6–8 days. The parents



Fig 2 Paroxysmal torticollis in case 7 at 9 months of age showing curvature of the trunk and extension of the right leg (Ink drawing from 8 mm movie)

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Recovery from 1 year to 5 years of age
Female preponderance?

of the condition according to this series and reports from the literature are shown in Table 1

In more severe cases (case 2) extension of the extremities on one side are also seen. This involvement of the extremities has not been reported before.

In some cases the onset of an attack is accompanied by distress and vomiting. The first attack usually occurs during the first year of life and we have even seen it during the first week (case 4). The age of recovery is from 1 year to 5 years according to Snyder (14). The duration of attacks varies from 10 min to several days.

The main diagnostic problem is the first attack appearing in a previously healthy infant. Torticollis in infancy may be the first sign of a posterior fossa tumor (6) which therefore must be ruled out especially when accompanied by vomiting.

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recognized the condition in the morning. At the onset the baby seemed in sweat and was irritable but showed no other signs of distress during the attacks. At examination during an attack the baby was at first seen sleeping supine with her head bent to the left. Awakening she bent her head to the right and retracted it. Slight curvature of the trunk but no change of the tone in her arms or legs was noticed. The position was accentuated when the girl was held upright.

The girl is now 8 months old and except for the regularly recurring attacks healthy and well developed.

Examinations. Physical and neurological examinations were normal. Roentgen studies of the spine, electroencephalography during an attack and echo-encephalography revealed no abnormalities. ENG: Normal caloric reactions.

DISCUSSION

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Fig. 1 Paroxysmal torticollis in case 2 at 8 months of age (a) to the left side and (b) at the next occasion to the right

is a left-sided sensorineural hearing loss. Several other relatives also have sensorineural hearing loss.

Pre- and neonatal history as well as psychomotor development was quite normal. From 3 months of age attacks were noted when the girl kept her head to one side (Fig. 1). At the same time her trunk was curved and her contralateral leg rigidly extended with plantar flexion of the foot (Fig. 2). This posture was most pronounced in upright and supine positions but inhibited when prone or crawling.

At the onset of some attacks the mother noticed that the girl's eyes rolled upwards. At one occasion when her neck was straightened rolling of her eyes occurred until her head was tilted again. No engagement of the face and mouth was noted.

The attacks were always recognized in the morning. Especially at the beginning of an attack the girl sometimes seemed unwell, irritated and drowsy.

The duration of the attacks varied from 3–12 hours. The attacks recurred very regularly once a month but from the age of 1 year the frequency gradually diminished. From 1.5 to 2.5 years of age she had about 7 attacks, some very mild but other severe and showing some additional signs. The latest attack was described as follows. She had a balance disturbance and often fell. She walked on a broad base, unsteady with short steps. She had only a slight torticollis and no affection of her trunk or leg. When moving she preferred to crawl with her head bent down. Her favourite position was crouching. An evident tremor of grasping was noticed. Her eyes were seen rolling up at occasions. During the latest attack lasting 17 hours she refused to eat and drink. She vomited twice and a few times she complained of headache. This attack as the others stopped suddenly as a light button is switched off. She then could walk, drink, eat and play as usual. The girl is now 2.5 years old and quite healthy and well developed.

Examinations. Physical and neurological examinations never revealed any abnormalities. Ophthalmological examination, electroencephalography and echocardiography were normal as was electrocardiography

during an attack. Chromatography of the urine showed no abnormal organic acids. Roentgen studies of the skull and cervical spine was normal. Caloric tests during attack and in a free interval showed normal reactions bilaterally registered by ENG. Hearing tests indicate normal hearing.

Case 3

M J Female. First and only child. The parents were not consanguineous. Her mother has a slight sensorineural hearing loss on one side. Pre- and neonatal history was normal. From 2 months of age attacks with the head tilted to the left and sometimes slight curvature of the trunk. During an attack the torticollis gradually appeared within 2 days and faded within 2 weeks. During the attacks the baby was quite well. The attacks occurred very regularly with a free interval of 14 ± 1 days. Gradually the attacks became less visible and were noticed only when she was tired. From 1.5 years of age no attacks were seen. The girl is now 2 years old, healthy and well developed.

Examinations. Physical and neurological examination was normal. Ophthalmological examination and electroencephalography during an attack showed normal results. Roentgen studies of the cervical spine revealed no abnormalities. Rotation test showed normal reactions. (The girl refused to cooperate in caloric tests.) Hearing tests were normal.

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A K B Female. First and only child of healthy parents who were not consanguineous. The prenatal period was normal. Because of a prolonged labour the child was delivered by vacuum extraction. The Apgar score was 8 at 1 min. Two hours after birth two short convulsion episodes were observed, otherwise the neonatal period was normal.

At 1 week of age a torticollis was noticed which disappeared within 1 week. Thereafter especially right-sided torticollis attacks reappeared regularly with a free interval of 16–19 days and a duration of 6–8 days. The parents

that all these children really suffered from a unilateral peripheral vestibular disorder. Apparently ENG was not used and admittedly it is not easy to properly evaluate nystagmus reactions to caloric stimuli in children without ENG. Technical errors would also be the most likely explanation of the remarkable observations by Koenigsberger et al. (10) of a child with complete absence of vestibular reactions to caloric stimuli but with a normal rotation test. Since both the caloric and rotation tests stimulate the lateral ampullae the results of these tests must agree.

To further support his theory of a labyrinthine origin to paroxysmal torticollis Snyder (14) reports reduced hearing in 4 cases. As he gives no information of the severity or type of hearing impairment apart from saying that there were no signs of otitis media it is impossible to assess the validity of these findings.

In our series consanguinity between the parents was not found. In one family (case 1) relatives with a history consistent with paroxysmal torticollis were found. In the cases hitherto reported with paroxysmal torticollis where the sex is stated, 13 out of 17 children have been females. This tendency of female preponderance and the possible occurrence of similarly affected cases in the family might indicate that genetic causative factors might be working.

However, paroxysmal torticollis in infancy remains a puzzling periodic condition with many unsolved questions. The site of dysfunction is probably the brain stem or the cerebellum. The paroxysmal character could be explained by vascular or metabolic disturbances.

It is important to recognize the condition because the parents can be reassured that the prognosis is good.

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REFERENCES

1. Brasser L. S. Benign paroxysmal vertigo of childhood. *Brain* 87: 141, 1964.
2. Casteels Van Daele M. Paroxysmal torticollis in infancy. *Am J Dis Child* 120: 88, 1970.
3. Chutorian A. M. Benign paroxysmal torticollis tortipelvis and retrocollis in infancy. *American Academy of Neurology*. Twenty sixth Annual Meeting 1974.
4. Crammer J. L. Periodic diseases. Rhythm in medicine. *Documenta Geigy* 1965.
5. Ekblom K. A. Westerberg C. E. & Osterman P. O. Focal sensorymotor seizures of spinal origin. *Lancet* Jan 13: 67, 1968.
6. Fessard C. Cerebral tumors in infancy. *Am J Dis Child* 115: 30, 1968.
7. Golden G. S. & French J. H. Basilar artery migraine in young children. *Pediatrics* 56: 777, 1975.
8. Gourley I. M. Paroxysmal torticollis in infancy. *Can Med Assoc J* 105: 504, 1971.
9. Hennksson N. G. Pfaltz C. R. Torok N. & Rubin W. A. synopsis of the vestibular system. An effort to standardize vestibular conceptions, tests and their evaluation. *Sando Monographs* 1972.
10. Koenigsberger M. R. Chutorian A. M. Gold A. M. & Schvey M. M. Benign paroxysmal vertigo in childhood. *Neurology (Minneapolis)* 20: 1108, 1970.
11. Kyllerman M. & Steen G. Intermittently progressive dyskinetic syndrome in glutaric aciduria. *Neuropediatrics* 8: 397, 1977.
12. Nilsson S. E. & Floderus M. Nine cases of hereditary and nonhereditary periodic diseases. *Acta Med Scand* 175: 341, 1964.
13. Sanner G. Bergstrom B. & Kadesjo M. Benign paroxysmal infantile torticollis. 18th Scandinavian Pediatric Congress. Aarhus 1976.
14. Snyder C. H. Paroxysmal torticollis in infancy. *Am J Dis Child* 117: 458, 1969.
15. Sutcliffe J. Torsion spasm and abnormal postures in children with hiatus hernia. Sandifer's syndrome. *Pediatrics* 190: 190, 1969.
16. Tassman C. A. & Fine R. D. Paroxysmal choreoathetosis. *Proc Aust Assoc Neurol* 6: 71, 1969.

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drug could not prevent further attacks in our cases.

Cisteels Van Diele (2) stressed that paroxysmal torticollis could be a dystonic side reaction of phenothiazine therapy. However, this type of dystonia also involves the face and mouth, which distinguishes it from the condition of paroxysmal torticollis.

A dystonic headposture also has been reported in association with hirsutia hernia (15). In paroxysmal torticollis roentgen studies of the intestine have shown normal results (3). Furthermore, this dystonic reaction is mostly seen in males which was not the case in our patients. A dystonic condition with a paroxysmal tendency has recently been described in an organic acid disorder (11). Therefore, in our sample was collected during the day of an attack in case 2, but when analyzed, no abnormal organic acids could be found.

The above mentioned differential diagnoses might be considered in certain cases. However, they do not agree with the regular periodicity of paroxysmal torticollis which seems to be a cardinal feature. In 3 of our cases the free interval between attacks was about 14 days and in one case 1 month. Mostly the parents could tell almost exactly when the next attack was to appear. This is very striking and difficult to explain.

In other diseases with regular periodicity the intervals often have been 7-14 or further multiples of 7 days (4). A similar pattern is seen in our series too. Periodic diseases of this type are usually inherited (12).

The main feature of paroxysmal torticollis is the head tilting seen in all cases during infancy. The fact that the abnormal posture is altered in different positions (case 2) may suggest that it is influenced by postural reflex systems. Nevertheless, it is quite clear that the clinical features of paroxysmal torticollis as they appear in infancy are influenced by the degree of maturation of the central nervous system as the condition alters character with increasing age. This is exemplified by our case 2 where the attacks at 2 years of age more

and more were characterized by paroxysmal nystagmus with intention tremor and unsteady broad-based gait.

The condition seen in case 2 rather points to the possibility of a paroxysmal dysfunction of the cerebellum or the vestibulo-cerebellar connections. Theoretically, a paroxysmal insufficiency of the basilar artery supplying these areas could produce this picture which is not unlike basilar artery migraine (7). However, in migraine the attacks are shorter than those of paroxysmal torticollis usually are. Furthermore, in our series no child has got a paroxysmal headache and no close relatives are affected by migraine.

Snyder (16) compared the torticollis to Meniere's syndrome in the adult and also suggested that it was identical with the syndrome of benign paroxysmal vertigo in childhood reported by Basser (1). Although Meniere's disease may occur in children, there are no indications of a peripheral vestibular disorder as the cause of paroxysmal torticollis. Ataxia which Snyder mentions as a proof of vestibular disorder is not a vestibular symptom. Nine of Snyder's 12 cases and the case reported by Gourley (8) were said to have negative caloric reactions bilaterally. To elicit a perception of dysequilibrium from the peripheral vestibular system there must be an imbalance between impulses from the right and left side (9). Loss of both labyrinths causes some balance problems but no vertigo. In fact, one method of treatment in cases of bilateral Meniere's disease is streptomycin in vestibulotoxic doses to eliminate the vestibular sensory epithelia in both ears.

The same objection regarding the interpretation of the vestibular tests applies to the reports of Basser (1) and Koenigsberger et al (10) on paroxysmal vertigo in children. Basser states that the predominant finding is moderate to complete canal paresis which may be unilateral or bilateral. However, in the 8 cases who were said to have bilateral canal paresis it was more pronounced on one side than the other. It therefore seems probable

MYOTONIA CONGENITA (THOMSEN'S DISEASE)

Early Diagnosis in Infancy

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ABSTRACT Harel S. Chui, A. L. and Shapira Y. (Neuromuscular Unit, Department of Neurology, Los Angeles County University of Southern California Medical Center, Los Angeles, California) Myotonia congenita (Thomsen's disease). *Acta Paediatr Scand* 68 225 1979.—A family with myotonia congenita (Thomsen's disease) is reported in which the father and his two offspring are affected. The course was characterized by the early onset of clinical manifestations in both the father and his two children. In one child a clinical and electrical diagnosis of the disease was made as early as two weeks of age. Early manifestations were breathing difficulty and eye closure myotonia. The importance of early recognition of the disorder is emphasized.

KEY WORDS Myotonia, Thomsen's disease, congenital stridor.

Myotonia congenita (Thomsen's disease) is considered to be a relatively benign disorder characterized clinically by diffuse hypertrophy of voluntary muscles with widespread myotonia usually accentuated by rest and cold and relieved by exercise. The usual mode of inheritance is autosomal dominant but autosomal recessive inheritance has also been reported (1, 5, 9, 10). Symptoms are generally not apparent until childhood or adolescence although early symptoms of feeding difficulty and peculiar cry have been described (2, 12). Early diagnosis is uncommon and attention to such symptoms may lead to more frequent diagnosis in very young children. In this paper we report a father and his two offspring who presented with unusual transient respiratory stridor as early symptoms of myotonia. We believe that this is one of the earliest confirmed diagnoses of myotonia congenita reported.

REPORT OF CASES

Case 1 (D. J.) A 8 year-old Caucasian male was referred for evaluation of his myotonic condition. His chief complaint was that of occasional episodes of muscle stiffness and cramp involving the face and extremities. His illness dated back to the neonatal period at which time he had several episodes of respiratory difficulty with

cyanosis and syncope which were attributed to mechanical airway obstruction. At 8 months he developed difficulty in relaxing periorbital and masticatory muscles. Later these difficulties progressed to affect both upper and lower extremities. At two years the diagnosis of myotonia congenita was first made. The patient claimed to be absolutely symptom free between episodes of myotonia. His muscle symptoms occurred only immediately prior to or subsequent to febrile illnesses. Only at these times does he describe difficulty opening his eyes and fists, chewing solid foods as well as cramping pain in his arms and legs. He had no history of muscle weakness or intermittent attacks of paralysis.

Physical examination revealed a well developed extremely athletic looking man. Pertinent neurological findings were confined to the musculature. He showed generalized muscular hypertrophy most pronounced in the thenar and hypothenar eminences bilaterally. Muscle strength and deep tendon reflexes in all extremities were normal. He had percussion myotonia of the thenar eminences but no myotonia of the tongue could be elicited. Active myotonia characterized by slow opening of the clenched fist and difficulty opening his eyes in forced closure of eyelids was observed. In some instances the myotonia became progressively more severe with repeated movements (paradoxical myotonia) and on other occasions the symptoms were partially relieved by exercise. This observed pattern was consistent with the patient's own past experience. Serum electrolytes during episodes of myotonia and at other times were within normal limits. Electromyographic examination of the right thenar eminence muscle disclosed typical myotonic discharge.

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youngest patient this was easily done at the age of two weeks

Thus when confronted with a normal appearing and developing neonate with episodic feeding difficulty respiratory distress peculiar cry and eye closure myotonia the diagnosis of myotonia congenita should be considered even with an apparently negative family history since autosomal recessive inheritance is not uncommon (1 5 6 9 11) A brief EMG can then confirm the diagnosis

We feel that early recognition of the disease is imperative for differentiation from other life threatening conditions appropriate genetic counselling reassurance of the parents as to the benign course of the disease and possible early symptomatic relief with medication such as diphenylhydantoin (8)

REFERENCES

- 1 Becker P E *Proc 3rd National Congress Human Genetics* Chicago 1966
- 2 Bell D B & Smith D W Myotonic dystrophy in the neonate *J Pediatrics* 81 83 1972
- 3 Bell J Dystrophie myotonique and allied diseases *The Treasury Human Inheritance* London 4 343 1947

- 4 Dodge P R Gamstorp I & Byers R K Myotonic dystrophy in infancy and childhood *Pediatrics* 35 3 1965
- 5 Harper P S & Dylken H R Early onset dystrophie myotonique *Lancet* II 53 1972
- 6 Kirby J F & Kraft G H Electromyographic studies in myotonia congenita *Arch Phys Med Rehabil* 54 47 1973
- 7 Klein D La dystrophie myotonique (Steinert) et la myotonie congenitale (Thomsen) en Suisse *J Genet Hum* 7 Suppl P 1958
- 8 Munsat T L Therapy of myotonia A double blind evaluation of diphenylhydantoin procainamide and placebo *Neurology* 17 359 1967
- 9 Penders C A & Delwaide P J Formes cliniques de la myotonie congenitale none dystrophique *Acta Neurol Belg* 72 366 1972
- 10 Swarman H F & Wright F S *Neuromuscular diseases of infancy and childhood* Charles C Thomas Springfield Ill 1970 p 197
- 11 Thomsen E Myotonia Thomsen's Disease Iara myotonia and Dystrophie Myotonia *Op Ex Domo Biol Hered Hum* Copenhagen E Munksgaard 1948 vol 43
- 12 Walton J N *Disorders of voluntary muscle* Churchill Livingstone London 1974 p 479

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Case 2 (J D) the first child of Case 1 was first evaluated at the age of two months. The child's perinatal history was unremarkable. Early developmental milestones were normal. He was a very alert and socially responsive baby who began smiling at the age of six weeks. Because of the father's disease, the parents had been watching the baby closely and reported that when the baby cried he had transient episodes of difficulty in opening his eyes, the eyelids remaining closed for a few seconds. They also mentioned that on several occasions the baby had noisy inspiratory type breathing (also described as "losing his breath") for very short periods of time. During these episodes no change in skin color was noted. No feeding difficulty was reported.

On examination the baby was a bright alert strong normally moving two month old. No dysplastic features were seen. Occasionally and especially when crying he had short episodes of stridor accompanied by intercostal and subcostal retractions. His eyelids went into spasm and became difficult to open after crying. The cranial nerves were otherwise intact. Motor system examination revealed normally developed musculature with normal strength and tone. Deep tendon reflexes were normal. No myotonic response to muscle percussion could be elicited. Sensory examination and primitive reflexes were normal. Electromyographic examination using concentric needle electrodes was performed in his right deltoid, first dorsal interosseous, quadriceps, anterior tibialis and gastrocnemius muscles. Shortly after the insertion of the exploring needle electrode, high frequency waning and waxing positive potentials, identified as myotonic discharges, were seen constantly in all muscles tested. The motor unit profile on minimum and maximum contraction was normal.

The baby was seen in follow up at two years of age. His psychomotor development was normal. He still had episodes of breathing difficulty ranging in frequency from several times per day to one or two times per week. While crying he continued to have difficulty with relaxation and opening of his eyes.

Case 3 (G J) the second child of D J was first evaluated at the age of two weeks. She was born after an uncomplicated pregnancy and delivery. Her mother observed that the baby had a peculiar cry with noisy inspiratory breathing similar to her older brother but there were no eyelid or generalized myotonic symptoms. On examination the baby showed a normal developmental pattern. There was no evidence of percussion or grip myotonia. When crying she demonstrated short episodes of inspiratory stridor without cyanosis. All primitive reflexes were present.

A brief electromyographic examination was performed on her right first dorsal interosseous, anterior tibialis and gastrocnemius muscles. Typical myotonic discharges were observed in all muscles tested. The motor unit parameters were normal.

DISCUSSION

The clinical course of the disease was characterized by the early onset of symptomato-

logy in all three cases. All experienced breathing difficulties in the neonatal period. The appearance of eye closure myotonia was somewhat delayed in the father until about eight months of age. Although Thomsen's disease is known to be present from birth, symptoms are rarely apparent until late infancy or childhood in most reported cases and in our personal experience. Affected infants have occasionally been described with symptoms such as strangled cry or feeding difficulties (2, 3). Although pulmonary disability with emphysema and so called myotonic asthma has been described in older patients (17), neonatal respiratory symptoms have not been reported to our knowledge. Despite some similarity, the differentiation of Thomsen's disease from myotonic dystrophy in the neonate is possible. In the latter disease the clinical picture is much more severe and may include profound hypotonia, weakness, fasciculation, cranial dysmorphism, decrease in muscle mass, poor feeding, respiratory distress, talipes equinovarus, cryptorchidism and hypomineralized ribs and long bones (2, 4, 7).

Although electrical myotonia was present in all three cases, the children showed no mechanically induced myotonia and in the father the myotonia was present only in the thenar eminence. The inconsistent response of the father's myotonia to exercise differs somewhat from the usual improvement of myotonia after exercise. Myotonia is usually accentuated by cold but was induced in the father only by fever in the course of febrile illnesses.

Myotonia congenita can present as a polymorphous picture with varying clinical manifestations (5, 9). Contrary to the clinical picture, the electromyographic findings in myotonia congenita are very consistent. Many are still for obvious reasons reluctant to perform electromyographic studies on young infants but in Thomsen's disease myotonia is widespread and the myotonic response to electrical stimulation is so typical that one can perform a brief EMG on only a few muscles for confirmation of the diagnosis. In our

EARLY DETECTION OF PRESCHOOL HEALTH PROBLEMS—ROLE OF PERINATAL RISK FACTORS

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ABSTRACT Köhler L, Svenningsen N W and Lindquist B (Department of Paediatrics, University Hospital, Lund, Sweden). Early detection of preschool health problems—role of perinatal risk factors. *Acta Paediatr Scand* 68 229 1979.—To evaluate a perinatal risk grouping system 1267 4-year-old children went through a comprehensive health examination. A total of 41.5% of the newborns were included in the wide criteria of risk, which were more common among boys and among children of the youngest and oldest mothers. Among the 4-year-old the frequency of significant physical health problems was 15.8% including 10.1% visual disturbances and 2% neurological disorders. In some combinations of riskgroups and later health problems there were statistically significant correlations, e.g. regarding prematurity and cerebral irritation vs. cerebral palsy, but not sufficient to serve its purpose as a screening instrument. Even the accumulation of especially serious events in the perinatal period gave no clue in later neurological disorders. The addition of low socio-economic status as a perinatal risk did not influence the outcome either. The reasons for the weak correlation between perinatal risk factors and later outcome of health disorders and handicaps are discussed, and it is concluded that to detect children with health problems there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children, including clinical examinations and screening procedures by well-trained personnel.

KEY WORDS Children at risk, preschool health problems, neurological disorders, cerebral palsy, visual disturbances, behaviour problems.

Preventive paediatrics in Sweden is provided by the Child Health Centres. According to regulations these centres should perform health examinations of the children's physical, mental and social development from birth until they start school at the age of seven years. The main purpose of this close health surveillance is to ensure an early recognition of disabling conditions and handicapping disorders. By the introduction of a special and elaborate health examination of all 4-year-old children a still more efficient tool in this early detection programme was created, leading to the finding of a reasonable number of previously unknown important deviations, especially by screening examinations of vision, hearing, bacteriuria and behaviour (12-17).

It was concluded that a thorough paediatric examination at this age revealed rather few children with unknown functionally important health problems, especially when compared with the time-consuming and expensive meth-

ods of examination, and that other ways of early detection of children with physical health problems should be investigated (17).

The interest was then directed towards the concept of children at risk, i.e. to select by some criteria at a very early age children specially liable to later develop health problems. Usually such criteria are based on gestational and neonatal events (29-32) but other factors should also be considered, e.g. social and environmental ones (22-28).

The purpose of the present study was to evaluate such a risk grouping system of newborns by examining the children at the age of 4 years.

MATERIAL

In two consecutive years 774 children belonging to the community of Lund were born in the maternity ward, University Hospital, Lund. Four years later 1.8% of these children were still resident in Lund and 1.6% of them were given a comprehensive health examination.

Table 2 Classification of health problems

Groups 2 and 3 are termed significant health problems (see text)

Method of examination	Group 0 Healthy child	Group 1 Slight deviation without importance	Group 2 Moderate deviation Treatment indicated	Group 3 Definitely handicapping disorder
General medical examination or an immedial data		Flat feet phimosi slight eczema	Minimal brain dysfunction retentio testis urinary tract infection	Cerebral palsy organic heart disease severe epilepsy juvenile rheumatic arthritis Amblyopia strabismus
Vision examination		Myopia ≤ -1.0 D Hyperopia $+2.75-3.75$ D Astigmatism ≤ -1.5 d $\times 0^\circ$ or ≤ -1.0 D $\times 90^\circ$	Myopia > -1.0 D Hyperopia $\geq +4$ D Astigmatism ≥ -0.5 D $\times 0^\circ$ or ≥ -1.5 D $\times 90^\circ$ Anisometropia ≥ 1.0 D	
Auditory examination		Mild otitis media cured by simple otological measures and few visits to the physician	Protracted otitis media requiring more intense therapy (adenoidectomy drainage tubes) Moderate sensorineural hearing impairment with need for follow up and control	Severe impairment requiring hearing aid or special education (training)

oldest mothers and among single mothers neonatal reasons were rarest among the young est mothers

The findings of important health problems among the children at the age of 4 years are shown in Table 5. The total percentage of significant physical health problems was

15.8% which is the same as found in the larger health examination performed some years previously 16.6% ($p > 0.05$) (15).

There was little difference between sub groups neurological disorders in this study 2.0% versus 2.8% previously visual disorders 10.1% vs 9.0% auditory disorders 2.5% vs 1.7% ($p > 0.05$) (13-15).

The somatic health problems are specified in Table 6. The prevalence of different neurological disorders was also similar between surveys minimal brain dysfunction (hyperactivity and distractibility combined with general clumsiness but without additional neurological signs) 0.9% vs 1.1% cerebral palsy 0.4% vs 0.4% psychomotor retardation 0.6% vs 0.3% and epilepsy 0.1% vs 0.1% ($p > 0.05$).

Considerable behaviour problems were noted by the parents less frequently in this study 7.4% vs 10.2% in the previous one ($p < 0.01$) (17).

Enuresis more than two nights a week was equally frequent 9.3% vs 8.7% ($p > 0.05$).

Table 7 shows the connection between risk factors and health problems at 4 years of age.

It is evident from this table that rather few differences in health problems existed at the

Table 3 Classification of the participants in a screening programme

Screening results	Diagnosis		
	Diseased	Non diseased	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

$$\text{Sensitivity} = \frac{a}{a+c} \times 100$$

$$\text{Specificity} = \frac{d}{b+d} \times 100$$

$$\text{Positive predictive value} = \frac{a}{a+b} \times 100$$

$$\text{Youden index} = \text{sensitivity} + \text{specificity} - 100$$

A Youden index of 50% or less means a screening test no better than random chance (%)

Table 1 *Newborn children at risk***A Genetic reasons**

Family history of diseases in eyes, ears or nervous system

B Prenatal reasons (disorders by the mother)

- 1 Endocrine disorders e.g. diabetes, thyroid disturbances
- 2 Disorders in the circulatory system e.g. heart, lungs or blood
- 3 Infectious diseases e.g. rubella or other virus infections (in first 16 weeks of pregnancy), lues, toxoplasmosis, pyelitis, severe bacterial infection (meningitis, sepsis)
- 4 Complications of pregnancy e.g. toxemia, imminent abortion, repeated or large vaginal haemorrhages
- 5 Drugs in repeated doses e.g. chemotherapy, antibiotics, cytostatics, hormones, hypnotics & ray other than chest & ray major surgery in early months of pregnancy
Preventive drugs such as vitamins and iron given *lege artis* are not registered
- 6 Psychiatric illness

C Perinatal reasons

- 1 Prolonged or difficult labour e.g. by instrument, breech presentation, primiparae >35 years of age, multiple birth, early rupture of membranes (more than 12 hours before partus), prolapse of umbilical cord
- 2 Disorders of the placenta e.g. placenta previa, abruption or placenta with many infarcts, deficiencies or larger haemorrhages in the placenta, Weight of placenta >900 g and <300 g by relatively normal birth weight in relation to gestational age. Only one umbilical artery
- 3 Short gestation (<36 weeks) and/or low birth weight (<2200 g) (i.e. prematurity in the present study)
- 4 Intra- or extruterine asphyxia (slow, irregular foetal heart rate with or without meconium stained fluid, Apgar score 0-6 at 1, 5 or 10 min. Also neonatal distress during the following days (respiratory insufficiency with apnoea or hyaline membrane disease, neonatal hypoglycaemia)

D Neonatal reasons

- 1 Cerebral irritation e.g. convulsions, hyperexcitability, hypertonia, other signs of pathological nervous findings
- 2 Low vitality e.g. obvious feeding difficulties, weight loss >15%, floppy infant syndrome
- 3 Jaundice leading to exchange therapy (2)
- 4 Malformations as listed by the National Board of Health and Welfare (11)
- 5 Dysplastic children e.g. low set ears
- 6 Other manifest or suspected abnormalities e.g. too wide or too narrow cranial sutures, strange smell, cry or look

METHODS

In the maternity ward, all newborn children were examined by an experienced neonatologist (NWS) on their first or second day of life according to a standardized scheme. As well as a physical examination and test of neonatal

reflexes, the degree of maturity was also evaluated, assessment of external features, neurological development and head circumference (4). The physical and neurological examinations were repeated on the day of discharge from the hospital, i.e. usually on day 5. Child fulfilling the criteria of risk-children (Table 1) was listed and the record was kept separately. Children needing immediate transfer to the neonatal intensive care unit were included in the risk register after discharge from the hospital.

The routine examinations and health surveillance within the Child Health Services were performed as usual; children needing medical attention were taken care according to prevailing routines.

At the age of 4 years, the children passed a comprehensive health examination including a thorough physical check up by an experienced paediatrician (L.K.), screening of vision, hearing, bacteriuria and behaviour problems. Children with health problems were referred further evaluation by respective specialists at the University Hospital of Lund. Details of these procedures have been published previously (16). The examination was supplemented with questionnaires to the parents regarding the child's development, previous and present health problems, and the family's social and educational standard. A 3 graded socio-economic grouping system widely used in Sweden was also employed (3). This system is based on paternal occupation, group 1 representing the high group. The records of children already under professional care were checked but otherwise, the information of the parents was unconfirmed. The existing records of those 20 children who failed to appear at the health examination were checked at the Department of Paediatrics, which is the main centre caring for sick and handicapped children in this area. Diseases and handicaps of these children were included in the final calculation of morbidity and perinatal risks.

After the professional evaluation of the health problem, an attempt was made to classify them according to the severity or importance for the child's wellbeing (Table 1). Deviations in groups 2 and 3 were regarded as functionally important health problems and were termed significant, while the slight deviations were termed insignificant (16).

Chi square analyses were used with Yates' correction where applicable.

In evaluating the screening procedures, definitions according to Table 3 were used (5).

RESULTS

The criteria for risk grouping were very wide and a total of 41.5% of the newborns were included with a small preponderance of boys 46.9% vs. 36.7% for girls. The distribution of various risk groups in relation to the mothers' age, marital status and socio-economic standard is shown in Table 4. Perinatal reasons were more common among the youngest and the

Socio-economic group	Statistical differences risk-no risk	
	II	III
0	0	100
1	31.7	31.2
2	33.3	33.3
3	33.3	33.3
4	39.5	6.0
5	39.5	36.8
6	44.4	33.3
7	40.0	40.0
8	3.8	37.5
9	31.8	38.4
10	30.6	38.9
11	28.7	46.4
12	3.8	34.3
13	34.6	3.7
14	41.9	39.0
15	33.3	3.1
16	10.0	0.0
17	41.8	31
18		66.7
19	18.9	6.4
20	31.4	34.0
21	29.8	3.6

DISCUSSION

There is a general agreement among paediatricians that early detection of most handicapping disorders makes the treatment easier and the results better. Since many of the most serious and disabling conditions are retrospectively connected with adverse events during pregnancy and neonatal period (9-19, 20) the concept of risk groups theoretically seems to be an attractive idea.

However in spite of considerable enthusiasm for the idea especially in Great Britain (29) the results of most studies have been disappointing. Not only did these risk groups come to include merely a part of the children with subsequent handicaps (6, 10, 21, 26) but they also included a lot of children who grew up to be perfectly normal (23, 31). Furthermore mild perinatal complications could have extensive effects and high risk conditions may survive without signs of damage (25). Besides the procedure of labelling and following up of

certain children may influence their development and affect the parent-child relationship (25).

It is evident that there are great methodological difficulties in studies of this kind: the sampling of patients studied, the selection and assessment of risk criteria and the evaluation of the follow up result. Problems of this kind are of course bound to happen especially when a great number of midwives and doctors with differing backgrounds, training and equipment examine and assess the children. Therefore it was thought that a strict scheme and standardized examinations performed by the same welltrained staff on a group of children selected only by their place of residence could make it possible to overcome some of these problems. Since the children were followed by the routine health examinations in the ordinary health surveillance system without access to the risk grouping the effect of labelling was thought to be minimal if any.

It is evident that too many children were contained in the risk register but the purpose was to include every known or suspected risk factor in a way that made it possible to select factors with the greatest impact on the children's subsequent health.

At 4 years of age the children were very

Table 6 Specification of significant somatic health problems among 1282 4 year old children

	n	%
Neurological disorders	6	2.0
Minimal brain dysfunction	17	0.9
Cerebral palsy	5	0.4
Psycho-motor retardation	7	0.6
Epilepsy		0.1
Other somatic disorders	15	1.1
Gigantism	1	0.1
Organic heart disease	4	0.3
Diabetes	1	0.1
Urinary tract infection	1	0.1
Haemophilia A	1	0.1
Reiteno testis	5	0.4
Congenital hip dislocation	1	0.1
Juvenile rheumatic arthritis	1	0.1
Total	41	3.2

Table 4 Distribution of 1282 risk grouped children on the mothers age, marital status and economic group (percentages)

Risk groups	Mothers				Statistical difference risk - no risk	Marital status		Statistical difference risk - no risk
	Age in years					Married	Single	
	<19	20-29	30-39	40+				
A Genetic reasons (n=1)	0	100	0	0		100	0	
B Prenatal reasons (n=197)	6.1	67.6	24.9	1.5		89.4	10.6	
1 Endocrine (n=9)	11.1	77.8	11.1	0		88.9	11.1	
2 Circulatory (n=30)	6.7	70.0	23.3	0		96.7	3.3	
3 Infections (n=95)	5.3	70.5	23.2	1.1		90.6	9.4	
4 Pregn. compl. (n=75)	5.3	60.0	32.0	2.7		90.7	9.3	
5 Drugs (n=9)	22.2	66.7	11.1	0		66.7	33.3	
6 Psychiatric (n=7)	0	100	0	0		40.0	60.0	
C Perinatal reasons (n=258)	9.4	64.4	23.1	3.1		84.4	15.6	
1 Difficult labour (n=151)	7.9	61.0	25.8	5.3		90.1	9.9	
2 Placenta disorders (n=72)	8.3	63.9	26.4	1.4		81.9	18.1	
3 Prematurity (n=48)	7.7	74.3	7.7	10.3		82.1	17.9	
4 Asphyxia (n=68)	16.4	46.8	22.4	4.5		79.1	20.9	
D Neonatal reasons (n=210)	1.9	74.5	22.6	1.0		97.3	2.7	
1 Cerebral irritation (n=42)	4.9	80.5	14.7	0		87.8	12.2	
2 Low vitality (n=87)	1.2	69.1	28.4	1.2		90.1	9.9	
3 Jaundice (n=10)	0	60.0	40.0	0		100.0	0	
4 Malformations (n=16)	6.3	68.8	25.0	0		100.0	0	
5 Dysplasia (n=9)	0	66.7	33.3	0		100.0	0	
6 Other (n=72)	1.4	80.6	16.7	1.4		91.7	8.3	
Any risk (n=532)	6.1	68.0	23.9	2.0		89.1	11.9	
No risk (n=750)	6.7	67.9	24.0	1.4		91.5	9.5	

age of 4 years between children considered to be at risk and children not considered to be at risk in the newborn period. Although the dif-

Table 5 Health problems among 1262 four year old children

	<i>n</i>	%
Significant somatic health problems	41	3.2
Neurological disorders	26	2.0
Other somatic disorders	15	1.2
Significant visual disorders	130	10.1
Strabismus	42	3.2
Refractive errors only	88	6.9
Significant auditory disorders	37	2.5
Sensorineural	3	0.2
Conductive	29	2.3
Sum significant physical health problems	203	15.8
Problems with upbringing and behaviour	93	7.4
Considerable behaviour problems	117	9.3
Enuretic > two nights a week	17	1.0
Very late speech development		

ferences in some risk groups are statistically significant, they are in no instance high enough to serve as prognostic signs, i.e. the sensitivity as well as the positive predictive value of risk grouping as a screening method are low. Thus, e.g. in using prematurity as a screening test for later outcome of cerebral palsy, sensitivity will be 60%, the specificity 5%, the Youden index 57% and the positive predictive value 7.5%. The combination of factors (24) shown in Table 8 did not change the outcome of the screening. The corresponding figures for prematurity combined with cerebral irritation will be 40%, 99.8%, 40%, 39.8% respectively. Even the foetal depletion of supply (7/27) alone (number 4, Table 8) or in combination with neonatal (number 5a in Table 8) did not select neurological disorders.

The addition of low socio-economic status as perinatal risk group did not influence outcome either (Table 8).

	Socio-economic group		Statistical differences risk-no risk
	II	III	
	11	100	
	31.7	31	
4		33.3	
3	1.3	33.3	
II	29	6.0	
7	19.4	36.8	
	44.4	33.3	
	40.0	40.0	
7	3.8	37.4	
18	31.8	38.4	
16	30.6	38.9	
4	8.7	46.4	
8	3.8	44.3	
	35.6	37	
1	43.9	39.0	
16	33.3	37.1	
10	10.0	70.0	
40	43.8	31	
11	77	66.7	
47	38.9	6.4	
5	33.4	34.0	
6	9.8	3.6	

DISCUSSION

There is a general agreement among paediatricians that early detection of most handicapping disorders makes the treatment easier and the results better. Since many of the most serious and disabling conditions are retrospectively connected with adverse events during pregnancy and neonatal period (9-19) the concept of risk groups theoretically seems to be an attractive idea.

However in spite of considerable enthusiasm for the idea especially in Great Britain (29) the results of most studies have been disappointing. Not only did these risk groups come to include merely a part of the children with subsequent handicaps (6-10, 21-26) but they also included a lot of children who grew up to be perfectly normal (23-31). Furthermore mild perinatal complications could have extensive effects and high risk conditions may survive without signs of damage (25). Besides the procedure of labelling and following up of

certain children may influence their development and affect the parent-child relationship (25).

It is evident that there are great methodological difficulties in studies of this kind: the sampling of patients studied, the selection and assessment of risk criteria, and the evaluation of the follow up result. Problems of this kind are of course bound to happen especially when a great number of midwives and doctors with differing backgrounds, training and equipment examine and assess the children. Therefore it was thought that a strict scheme and standardized examinations performed by the same welltrained staff on a group of children selected only by their place of residence could make it possible to overcome some of these problems. Since the children were followed by the routine health examinations in the ordinary health surveillance system without access to the risk grouping the effect of labelling was thought to be minimal if any.

It is evident that too many children were contained in the risk register but the purpose was to include every known or suspected risk factor in a way that made it possible to select factors with the greatest impact on the children's subsequent health.

At 4 years of age the children were very

Table 6. Specification of significant somatic health problems among 1282 4-year old children

	n	%
Neurological disorders	76	7.0
Minimal brain dysfunction	12	0.9
Cerebral palsy	5	0.4
Psycho-motor retardation	7	0.6
Epilepsy	7	0.1
Other somatic disorders	15	1.1
Gigantism	1	0.1
Organic heart disease	4	0.3
Diabetes	1	0.1
Urinary tract infection	1	0.1
Haemophilia A	1	0.1
Retentio testis	5	0.4
Congenital hip dislocation	1	0.1
Juvenile rheumatic arthritis	1	0.1
Total	41	3.7

tology has been very rapid new electronic equipment for surveillance during pregnancy and labour has been produced new indications for instrumental deliveries have been applied wider criteria and more efficient use of assisted breathing of the newborn have come into practice. Also other definitions and terms are prevailing. A study starting too dry would of course include small for-date children and use a 24 hours limit for early rupture of membranes instead of 12 hours and set the border of low birth weight of 2500 g instead of 2200 g. This means that the risk factors used in this study to predict the outcome of too drys 4 year olds do not reflect the present situation of the newborns. However there is no other way of assessing the importance of perinatal events than to examine the children many years later. Maybe even 4 years is too short a time some of the health problems will not appear until greater demands on intellectual and perceptual functions are made on the children in school. A new study including a foetal monitoring of all the subjects is now going on and the children are to be followed to school age.

For the time being however there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children including clinical examinations and screening procedures by well trained personnel. For the parents obstetricians and neonatologists it may be of some satisfaction that children at risk according to traditional criteria do not develop more handicaps than other children.

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REFERENCES

- Alberman E D & Goldstein H The at risk register: A statistical evaluation *Br J Prev Soc Med* 24: 129 1970
- Avery G B *Neonatology: Pathophysiology and management of the newborn* J P Lippincott (Philadelphia) 1975 pp 347-350
- Election for the Riksdag* Central Bureau of Statistics Stockholm 1953
- Finnstrom O Studies on maturity in newborn infants. VI Comparison between different methods of maturity estimation *Acta Paediatr Scand* 61: 1972
- Frankenburg W K Pediatric screening. In: Schulman (ed) *Advances in pediatrics* vol 70 1c Book Medical Publishers Chicago 1973
- Hamilton F M W The early detection of handicap in children *Med Offr* 170: 167 1968
- Hagberg G, Hagberg B & Olow I The changing panorama of cerebral palsy in Sweden 1944-1976. The importance of foetal deprivation of supply *Acta Paediatr Scand* 65: 403 1976
- Hofsten E Social classes in Swedish statistics production *Statistisk Tidskrift* 5: 368 1961
- Illingworth R S The diagnosis of cerebral palsy: the first year of life *Develop Med Child Neurol* 8: 178 1966
- Knox E G & Mahon D F Evaluation of Infant's risk register *Arch Dis Child* 45: 634 1970
- Källén B & Winberg J A Swedish register of congenital malformations *Pediatrics* 41: 765 1968
- Kohler L, Fritz H & Schersten B Bacteriuria in 4 year old children *Acta Paediatr Scand* 61: 785 1972
- Kohler L & Holst H E Auditory screening of four year old children *Acta Paediatr Scand* 61: 555 1972
- Kohler L & Stigmar G Vision screening of four year old children *Acta Paediatr Scand* 67: 17 1973
- Kohler L Physical health of four year old children *Acta Paediatr Scand* 67: 181 1973
- Kohler L Health control of four year old children. An epidemiological study of child health *Acta Paediatr Scand Suppl* 735 1973
- Kohler E M & Kohler L Health and behaviour of four year old children *Acta Paediatr Scand* 64: 775 1975
- Kohler L Physical health of 7 year old children. An epidemiological study of school entrants and a comparison with preschool health *Acta Paediatr Scand* 66: 297 1977
- Lilienfeld A M & Pasamanick B The association of maternal and fetal factors with the development of cerebral palsy and epilepsy *Am J Obstet Gynecol* 70: 93 1955
- Little J W Lecture VIII in Course of lectures on the deformities of the human frame *Lancet* p 319 1843
- Mitchell M G Changing concept of risk *Develop Med Child Neurol* 17: 277 1975
- Nehigan G A, Kolvin I, Scott D Mcl & Garside R F Born too soon or born too small. A follow up study to seven years of age *Clinics in Developmental Medicine* No 61 Heinemann London 1976
- Oppe T E Risk registers for babies *Develop Med Child Neurol* 11: 167 1967

- 4 Parmelee A H & Haber A Who is the Risk infant? *Clinical Obstet Gynecol* 16 376 1973
- 5 Prechtl J F M Chairman's conclusion In Z K Stembera K Polacek & V Sabata (eds) *Perinatal medicine* Georg Thieme Stuttgart 1975
- 6 Rogers M G H The early recognition of handicapping disorders in childhood *Develop Med Child Neurol* 13 88 1971
- 7 Sabel K M Olegård R & Victorin L Remaining sequelae with modern perinatal care *Pediatrics* 57 65 1976
- 8 Sameroff A J Early influences on development—fact or fancy? *Merrill Palmer Quarterly of Behavior and Development* 21 167 1975
- 9 Sheridan M Infants at risk of handicapping conditions *Mon Bull Min Health* 21 138 196
- 10 Stratton H M Criteria for assessing the influence of obstetric circumstances on later development In T

- Chard & M Richards (eds) *Benefits and hazards of the new obstetrics* Clinics in Developmental Medicine No 64 William Heinemann London 1977
- 11 Thomson A J Searle M & Russell G Quality of survival after severe birth asphyxia *Arch Dis Child* 52 670 1977
- 12 Walker H G An assessment of the current status of the at risk register *Scottish Home and Health Department* 1967

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For the time being however there seems to be no acceptable alternative to a comprehensive health surveillance as part of a general health service programme of all children including clinical examinations and screening procedures by well trained personnel. For the parents obstetricians and neonatologists it may be of some satisfaction that children at risk according to traditional criteria do not develop more handicaps than other children.

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REFERENCES

- 1 Altherman E H & Goldstein H The at risk register. A statistical evaluation *Br J Prev Soc Med* 24 129 1970
- 2 Avery G B Neonatology Pathophysiology and management of the newborn J P Lippincott Co Philadelphia 1975 pp 347-340
- 3 Elections for the Riksdag Central Bureau of Statistics Stockholm 1953
- 4 Innström O Studies on maturity in newborn infants VI Comparison between different methods for maturity estimation *Acta Paediatr Scand* 61 1972
- 5 Frankenburg W K Pediatric screening In J Schulman (ed) *Advances in pediatrics* vol 70 Year Book Medical Publishers Chicago 1973
- 6 Hamilton F M W The early detection of handicap in children *Med Offr* 170 167 1968
- 7 Hagerberg G Hagerberg H & Olow I The changing panorama of cerebral palsy in Sweden 1946-1970 The importance of foetal deprivation of supply *Acta Paediatr Scand* 65 403 1976
- 8 Hofsten F Social classes in Swedish statistics production *Statistisk Tidskrift* 5 368 1967
- 9 Illingworth H S The diagnosis of cerebral palsy in the first year of life *Develop Med Child Neurol* 8 178 1966
- 10 Knox L G & Mahon D F Evaluation of infant at risk register *Arch Dis Child* 45 634 1970
- 11 Kallén B & Winberg J A Swedish register of congenital malformations *Pediatrics* 41 765 1968
- 12 Köhler I Fritz H & Scherstén B Bacteremia in 4 year old children *Acta Paediatr Scand* 61 789 1972
- 13 Köhler L & Holst H E Auditory screening of four year-old children *Acta Paediatr Scand* 61 455 1972
- 14 Köhler L & Stigmar G Vision screening of four year old children *Acta Paediatr Scand* 62 17 1973
- 15 Köhler L Physical health of four year-old children *Acta Paediatr Scand* 62 181 1973
- 16 Köhler L Health control of four year-old children. An epidemiological study of child health *Acta Paediatr Scand Suppl* 235 1973
- 17 Köhler I M & Köhler I Health and behaviour of four year old children *Acta Paediatr Scand* 64 275 1975
- 18 Köhler L Physical health of 7 year old children. An epidemiological study of school entrants and a comparison with preschool health *Acta Paediatr Scand* 66 297 1977
- 19 Lilienfeld A M & Passimnick B The association of maternal and fetal factors with the development of cerebral palsy and epilepsy *Am J Obstet Gynecol* 70 93 1955
- 20 Little J W Lecture VIII in Course of lectures on the deformities of the human frame *Lancet* p 319 1843
- 21 Mitchell R G Changing concept of risk *Develop Med Child Neurol* 17 277 1975
- 22 Nelson G A Kolvin I Scott D McI & Garside R F Born too soon or born too small. A follow up study to seven years of age. Clinics in Developmental Medicine No 61 Heinemann London 1976
- 23 Oppé T E Risk registers for babies *Develop Med Child Neurol* 13 1967

- 74 Parmelee A H & Haber A Who is the Risk infant? *Clinical Obstet Gynecol* 16: 376 1973
- 75 Prechtl H F R Chairman's conclusion In K. Stembera K. Polacek & V. Sabata (eds) *Prenatal medicine* Georg Thieme Stuttgart 1975
- 76 Rogers H H The early recognition of handicapping disorders in childhood *Develop Med Child Neurol* 13: 1971
- 77 Sabel K H Olegård H & Victorin L Remaining sequelae with modern prenatal care *Pediatrics* 57: 637 1976
- 78 Sameroff A J Early influences on development—fact or fancy? *Merrill Palmer Quarterly of Behavior and Development* 21: 67 1975
- 79 Shendan M Infants at risk of handicapping conditions *Mon Bull Min Health* 21: 238 1967
- 80 Stratton P M Criteria for assessing the influence of obstetric circumstances on later development In T

- Chard & M Richards (eds) *Benefits and hazards of the new obstetrics* Clinics in Developmental Medicine No 64 William Heinemann London 1977
- 81 Thomson A J Searle M & Russell M Quality of survival after severe birth asphyxia *Arch Dis Child* 52: 670 1977
- 82 Walker R G An assessment of the current status of the at risk register *Scottish Home and Health Department* 1967

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REFERENCES

- 1 Alberman E D & Goldstein H The at risk register. A statistical evaluation. *Br J Prev Soc Med* 24 129 1970
- 2 Avery G B *Neonatology Pathophysiology & management of the newborn* J P Lippincott Co Philadelphia 1975 pp 347-350
- 3 *Flecnons for Riksdag* Central Bureau of Statistics Stockholm 1953
- 4 Finnstrom O Studies on maturity in newborn infants VI Comparison between different methods for maturity estimation. *Acta Paediatr Scand* 61 33 1972
- 5 Frankenburg W K Pediatric screening. In J Schulman (ed) *Advances in pediatrics* vol 0 Year Book Medical Publishers Chicago 1973
- 6 Hamilton F M W The early detection of handicap in children. *Med Offr* 170 167 1968
- 7 Hagberg G Hagberg B & Olow I The changing panorama of cerebral palsy in Sweden 1954-1970 The importance of foetal deprivation of supply. *Acta Paediatr Scand* 65 403 1976
- 8 Hofsten E Social classes in Swedish statistics production. *Statistisk Tidskrift* 5 368 1967
- 9 Illingworth R S The diagnosis of cerebral palsy in the first year of life. *Develop Med Child Neurol* 8 178 1966
- 10 Knox E G & Mahon D F Evaluation of infant at risk register. *Arch Dis Child* 45 634 1970
- 11 Kallen B & Winberg J A Swedish register of congenital malformations. *Pediatrics* 41 765 1968
- 12 Kohler L Fritz H & Schersten B Bacteriuria in 4 year-old children. *Acta Paediatr Scand* 61 789 1972
- 13 Kohler L & Holst H E Auditory screening of four year-old children. *Acta Paediatr Scand* 61 555 1972
- 14 Kohler L & Stigmar G Vision screening of four year old children. *Acta Paediatr Scand* 62 17 1973
- 15 Kohler L Physical health of four year-old children. *Acta Paediatr Scand* 62 181 1973
- 16 Kohler L Health control of four year old children. An epidemiological study of child health. *Acta Paediatr Scand Suppl* 215 1973
- 17 Kohler E M & Kohler L Health and behaviour of four year old children. *Acta Paediatr Scand* 64 725 1975
- 18 Kohler L Physical health of 7 year-old children. An epidemiological study of school entrants and a comparison with preschool health. *Acta Paediatr Scand* 66 297 1977
- 19 Lilienfeld A M & Pasamanick H The association of maternal and fetal factors with the development of cerebral palsy and epilepsy. *Am J Obstet Gynecol* 70 93 1955
- 20 Little J W Lecture VIII in Course of lectures on the deformities of the human frame. *Lancet* p 319 1843
- 21 Mitchell R G Changing concept of risk. *Develop Med Child Neurol* 17 277 1975
- 22 Neligan G A Kolvin I Scott D McI & Garside H F *Born too soon or born too small. A follow up study to seven years of age* Clinics in Developmental Medicine No 61 Heinemann London 1976
- 23 Oppé T E Risk registers for babies. *Develop Med Child Neurol* 9 13 1967

PLASMA AND RED BLOOD CELL FOLATE IN BREASTFED INFANTS

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ABSTRACT Ek J and Magnus E M (Department of Paediatrics Rikshospitalet and Kroghstøtten Department of Oslo City Hospital Oslo Norway) Plasma and red blood cell folate in breastfed infants. *Acta Paediatr Scand* 68 239 1979. — We have studied growth red blood cell status and folate concentrations in plasma and red cells in a group of 35 breastfed infants during the first year of life. Folic acid supplementation was not given to the mothers during pregnancy or lactation and none of them developed megaloblastic anaemia. The growth and red blood cell status of the infants were both normal. At birth and throughout the period of observation the folate concentrations in plasma and red cells were significantly higher than in the adult reference material. A positive correlation between plasma and red cell folate was demonstrated. During the latter part of pregnancy and lactation the foetuses and infants seem to be protected against folate deficiency. We regard the folate status of normal breastfed infants as optimal. The optimal supply of the vitamin in artificial nutrition should be the amount of folate necessary to maintain plasma and red cell folate concentrations similar to those found in breastfed infants.

KEY WORDS Infants breastfeeding plasma folate red blood cell folate red blood cell status

Folate is necessary for normal growth (4, 6) development (1) and function (2, 28) of the human organism. A prospective study on the folate status of infants and children related to other pertinent parameters was therefore undertaken. If possible we wanted to define the optimal daily supply of the vitamin. This is the first report from a series of studies made in a developed urban community. We concentrate here on the folate levels in breastfed infants and demonstrate that the infants seem to be protected against folate deficiency during the nursing period. Data illustrating the effects of normal physiological processes on plasma and red blood cell folate during the first year of life are also presented.

MATERIAL AND METHODS

The material in this study consists of 35 full term infants borne by healthy mothers from the socio-economic middle class. The mothers were given 10 mg iron daily from the third month of gestation but no folic acid supplementa-

tion. None of them developed anaemia during pregnancy or lactation.

The infants were breastfed for at least 6 months and up to 14 months, mean sucking period 7½ months. After weaning the infants were fed unboiled pasteurized cow's milk. Vegetables, iron fortified cereals containing 10–5 mg or more Fe per 100 g cereal meat and fish were gradually introduced at the age of 3 to 3½ months. Vitamin supplementation with ascorbic acid (orange juice) and vitamin A and D (cod liver oil) was started one month after birth: initially 5 mg ascorbic acid, 3750 IU vitamin A and 375 IU vitamin D, increasing to 15 mg, 7500 IU and 750 IU respectively from 1½ months of age.

The infants were examined regularly by one of us (J. E.) 3–5 hours after their last meal. They were first seen within 7 days after birth, thereafter at monthly intervals (4–6 weeks). On each occasion a clinical examination was performed and a capillary blood sample drawn. The folate concentration in plasma and red blood cells was measured and the infants' haemoglobin, RBC, VPRC, red cell indices, WBC, reticulocytes and blood smears were studied.

The adult reference material consisted of 50 female (non pregnant, non lactating) and 50 male students and hospital staff, aged 19 to 50 (mean 27½) years. Venous blood samples were drawn 3–5 hours after their last meal.

The haemoglobin values were obtained by a manual oxyhaemoglobin method (7, 21), red and white blood cell counts by a Celscope 401* (Lanson Instrument AB).

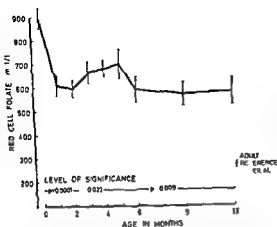


Fig 2 Red cell folate in 35 breastfed infants during first year of life. Mean values ± 1 S.E.M. Statistical differences in red cell folate levels at various ages are denoted by levels of significance

($r=0.38$ $n=34$ $p<0.05$) a positive correlation was further observed between the plasma folate concentration at 4 months of age and that of the red blood cell folate concentration at 6 months ($r=0.53$ $n=35$ $p=0.001$) at 9 months ($r=0.49$ $n=33$ $p<0.01$) and at 12 months of age ($r=0.35$ $n=34$ $p<0.05$).

No significant correlation was found between the haemoglobin concentration and the concentration of folate in plasma and red cells at the ages of 0, 3, 6, 9 and 12 months.

DISCUSSION

It is well established that the plasma and red cell folate concentrations are higher in newborn full term infants than in their mothers and in adult reference materials (5, 8, 13). The present study confirms those observations. Hamen & Caston (9) were able to demonstrate high concentrations of a folate binding protein in human umbilical cord serum different from that found in serum from pregnant women. The mechanism by which the foetus is able to accumulate folate against a concentration gradient at the expense of the mother may thus include the existence of different folate binders in mother and foetus.

During the whole nursing period the plasma and red cell folate values were higher than those recorded in the adult reference material. In Israel however Matoth et al (16, 17) found whole blood folate levels in breastfed infants to be of the same magnitude as in an adult reference material. The reason for this discrepancy is not clear. A folate binding protein is present in human milk in high concentrations (27). This protein may explain at least partly the high folate concentrations we have found in breastfed infants. The folate values for the mothers were even lower during the lactation period (5) than during pregnancy.

The infants were in negative folate balance during the first 2 months of life as judged from the decrease in red cell folate during that period. The infants then went into a period of positive folate balance. Landon & Oxley (13) observed a similar decrease in plasma folate during the first months of life irrespective of the initial folate concentrations but they did not state the type of feeding during that period. Matoth et al (16) did not observe any differences in whole blood folate levels at different ages in breastfed infants. They did not however observe the infants from birth nor did they follow the same group of infants at different ages. Several factors may contribute to the observed variations in folate levels such as low folate concentrations in colostrum as compared with mature milk (11), rapid tissue uptake (22) and reduced tubular reabsorption (12) in the newborn infant.

During the last part of the nursing period the folate values in plasma and red cells were lower than the preceding values probably due to either decreased intake or to increased demands. The change in infant feeding during this period is probably significant, a reduction in the relative importance of breast milk as foods containing less folate are introduced.

A positive correlation between the plasma and red blood cell folate has previously been shown in adults (14). Our study demonstrates that this relationship can also be found in infancy. The observed correlation between

Table 1 Red blood cell status in breastfed infants during first year of life expressed as mean values with S D

A R M = adult reference material S D = standard deviation

Age (months)	No of obs	Hb (g/dl)	RBC ($\times 10^{12}/l$)	VPRC (l/l)	MCV (fl)
0	34	17.7 \pm 1.62	5.53 \pm 0.50	0.578 \pm 0.060	104.7 \pm 8.9
1	35	14.2 \pm 1.37	4.53 \pm 0.49	0.417 \pm 0.041	92.7 \pm 8.2
2	35	12.1 \pm 0.91	3.96 \pm 0.38	0.356 \pm 0.027	90.4 \pm 7.6
3	35	12.0 \pm 0.80	4.15 \pm 0.34	0.357 \pm 0.022	86.6 \pm 6.0
4	35	12.2 \pm 0.71	4.32 \pm 0.48	0.363 \pm 0.023	84.2 \pm 6.8
5	35	12.3 \pm 0.75	4.47 \pm 0.45	0.370 \pm 0.020	83.5 \pm 6.6
6	35	12.1 \pm 0.74	4.36 \pm 0.47	0.367 \pm 0.022	84.9 \pm 9.1
9	34	12.4 \pm 0.77	4.60 \pm 0.43	0.373 \pm 0.021	91.0 \pm 7.6
12	34	12.7 \pm 0.80	4.68 \pm 0.44	0.390 \pm 0.022	81.9 \pm 6.7
A R M female	50	13.6 \pm 0.64	4.49 \pm 0.33	0.411 \pm 0.022	91.0 \pm 5.8
A R M male	50	15.4 \pm 0.62	4.95 \pm 0.33	0.457 \pm 0.021	91.6 \pm 5.3

34 observations

Sweden) and the VPRC by using a microhaematocrit centrifuge for 3 minutes at 9400 rev/min at a mean of 6300 g. Hepanized capillary tubes were used. The haematological analyses were controlled by means of DADE[®] CH 60TM Abnormal and Normal Haematology Reference Serum and red blood cell folate were determined as *Lactobacillus casei* activity (15).

All results were analysed by BMDP statistical computer programs (3). The difference between groups of observations was tested by the Mann-Whitney test.

RESULTS

The growth of the infants was normal and did not differ from the standards established for Norwegian (25) and Swedish infants (10).

The haematological data are presented in Table 1. The values found in the 50 adult males used as controls were compatible with those reported for Norwegian men by Natvig & Vellar (20). The reference values found in adult females were compatible with those reported in Norwegian women without iron supplementation (20).

The plasma folate concentrations (Fig. 1) were higher than in the adult reference material throughout the period of observation ($p < 0.001$). The red cell folate concentrations (Fig. 2) were also higher than in the adult reference material during the first year of life ($p < 0.001$).

The correlation between plasma and red blood cell folate was studied. A positive correlation was found between plasma and red

cell folate at the age of one month ($r = 0.40$, $n = 35$, $p < 0.05$) at 3 months ($r = 0.34$, $n = 35$, $p < 0.05$) at 5 months ($r = 0.63$, $n = 35$, $p < 0.001$) at 6 months ($r = 0.50$, $n = 35$, $p < 0.01$) and at 9 months ($r = 0.45$, $n = 33$, $p < 0.01$). A positive correlation was also observed between the plasma folate concentration at 5 months of age and that of the red blood cell folate concentration at 6 months ($r = 0.36$, $n = 35$, $p < 0.05$) as well as between the plasma folate at 9 months and that of red cell folate at 12 months of age.

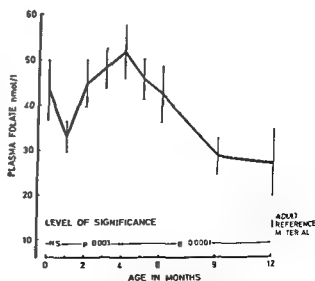


Fig. 1 Plasma folate in 35 breastfed infants during first year of life. Mean values \pm 1 S E M (Standard Error of the Mean). Statistical differences in plasma folate levels at various ages are denoted by levels of significance. NS = not significant.

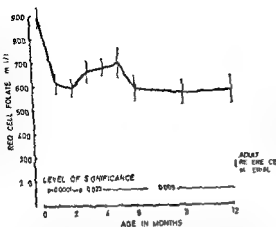


Fig. 2 Red cell folate in 35 breastfed infants during first year of life. Mean values \pm S.E.M. Statistical differences in red cell folate levels at various ages are denoted by levels of significance.

($r=0.38$, $n=34$, $p<0.05$) a positive correlation was further observed between the plasma folate concentration at 4 months of age and that of the red blood cell folate concentration at 6 months ($r=0.53$, $n=35$, $p=0.001$), at 9 months ($r=0.49$, $n=33$, $p<0.01$) and at 12 months of age ($r=0.35$, $n=34$, $p<0.03$).

No significant correlation was found between the haemoglobin concentration and the concentration of folate in plasma and red cells at the ages of 0, 3, 6, 9 and 12 months.

DISCUSSION

It is well established that the plasma and red cell folate concentrations are higher in newborn full term infants than in their mothers and in adult reference materials (4, 8, 13). The present study confirms those observations. Hamen & Caston (9) were able to demonstrate high concentrations of a folate binding protein in human umbilical cord serum different from that found in serum from pregnant women. The mechanism by which the foetus is able to accumulate folate against a concentration gradient at the expense of the mother may thus include the existence of different folate binders in mother and foetus.

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The infants were in negative folate balance during the first 2 months of life as judged from the decrease in red cell folate during that period. The infants then went into a period of positive folate balance. Landon & Orley (13) observed a similar decrease in plasma folate during the first months of life irrespective of the initial folate concentrations but they did not state the type of feeding during that period. Matoth et al (16) did not observe any differences in whole blood folate levels at different ages in breastfed infants. They did not however observe the infants from birth nor did they follow the same group of infants at different ages. Several factors may contribute to the observed variations in folate levels such as low folate concentrations in colostrum as compared with mature milk (11), rapid tissue uptake (22) and reduced tubular reabsorption (12) in the newborn infant.

During the last part of the nursing period the folate values in plasma and red cells were lower than the preceding values, probably due to either decreased intake or to increased demands. The change in infant feeding during this period is probably significant, a reduction in the relative importance of breast milk as foods containing less folate are introduced.

A positive correlation between the plasma and red blood cell folate has previously been shown in adults (14). Our study demonstrates that this relationship can also be found in infancy. The observed correlation between

plasma folate at one age and red cell folate later on implies that the red cell folate probably reflects the amount of folate available in the bone marrow for deposition in the red cell during the development of the cell.

Compared with the results reported by Moe (19) we found the haemoglobin concentration about 0.6 g/100 ml and the red blood cell count about $0.2 \times 10^{12}/l$ higher and the VPRC about 0.02 l/l lower than Moe. Methodological differences may well partially explain the differences Moe however found a mean MCV 7–12% higher than we did. This could indicate a slight tendency to megaloblastosis in his material. At the time of his study the milk for milk was not supplemented with folic acid.

During the last part of pregnancy and lactation there are mechanisms in function which within certain limits provide the foetus and suckling infant with folate at the expense of the mother. In this country nutritional folate deficiency in otherwise healthy women is very rare and folic acid supplementation is therefore not recommended. We believe that the folate status of women in Norway is adequate and that the foetuses and infants are usually assured an adequate supply.

The daily requirement of folate has been calculated from the folate content in human milk (16). This is an approach which may have certain limitations regarding artificial nutrition in infancy as we know that several nutrients are better utilized from human milk than from cow's milk preparations e.g. iron (18) and ascorbic acid (23). In a milk formula the amount of folate finally available for metabolic purposes will depend on factors such as the content of the vitamin in the raw material, the nature of the potentially supplemented vitamin, the effect of the manufacturing procedure, storage and preparation before use as well as on the biological availability and absorption.

A nutritional folate deficiency may well be present without any gross biochemical, physiological or morphological disturbances. The amount of the vitamin necessary to maintain

a normoblastic erythropoiesis (24–26) may therefore not be optimal.

It is reasonable to believe that an optimal tissue saturation of folate is present in normal breastfed infants providing the mothers' folate status is adequate. We suggest that the daily requirement during early infancy is the amount of folate necessary to maintain plasma and red cell folate concentrations similar to those found in breastfed infants.

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REFERENCES

1. Arakawa T, Mizuno T, Honda Y, Tamura T, Watanabe A, Komatsushiro M, Takagi T, Iinuma K & Yamuchi N. Longitudinal study of maturation patterns of EEG basic waves of infants fed on milk from mothers with low serum folate level. *Tohoku J Exp Med* 103: 81, 1970.
2. Davidson G P & Townley R R W. Structural and functional abnormalities of the small intestine due to nutritional folic acid deficiency in infancy. *Pediatrics* 90: 590, 1977.
3. Dixon W J. *BMDP Biomedical Computer Programs*. University of California Press, Berkeley, 1975.
4. Ek J. The clinical importance of folate deficiency in coeliac disease in infancy and childhood. In *Proc 15th Internat Congr Paediatr*, New Delhi, Oct 1977. Indian Academy of Pediatrics, Bombay. Abstr no 0607/03.
5. Ek J & Magnus E M. Folic acid levels in infancy. In *Proc 14th Internat Congr Paediatr*, Buenos Aires, Oct 3–9, 1974. Panamericana, Buenos Aires, 1974: 3–9.
6. Grady G & Jacobson W. Influence of folic acid on birthweight and growth of the erythroblastic infant. III. Effect of folic acid supplementation. *Arch Dis Child* 52: 16, 1977.
7. Harboe M. Studies on the conversion of oxyhaemoglobin to alkaline haematin in dilute alkaline solutions. *Scand J Clin Lab Invest* 11: 138, 1959.
8. Hibbard E D. Plasma and erythrocyte folate concentrations in normal mature infants. *Arch Dis Child* 48: 743, 1973.
9. Kamen B A & Caston J B. Purification of folate binding factor in normal umbilical cord serum. *Proc Natl Acad Sci USA* 72: 4761, 1975.
10. Karlberg P, Engstrom I, Lichtenstein H &

- Svennberg I The development of children in a Swedish urban community A prospective longitudinal study III Physical growth during the first three years of life *Acta Paediatr Scand* Suppl 187 48 1968
- 11 Karlén R Sur les variations du taux d'acide folique total dans le lait de femme au cours de la lactation Comparaison avec le lait bovin *C R Soc Biol (Paris)* 160 71 3 1966
- 12 Landon M J & Hey E N Renal loss of folate in the newborn infant *Arch Dis Child* 49 79 1974
- 13 Landon M J & Oxtley A Relation between maternal and infant blood folate activities *Arch Dis Child* 46 810 1971
- 14 Magnus E Folate studies *Scand J Haematol* Suppl 24 1975
- 15 Magnus E M In preparation
- 16 Matoth Y Pinkas A & Sroka Ch Studies on folic acid in infancy III Foliates in breast fed infants and their mothers *Am J Clin Nutr* 16 356 1965
- 17 Matoth Y Pinkas A Zamir R Moosalem F & Grossowicz N Studies on folic acid in infancy I Blood levels of folic and folic acid in healthy infants *Pediatrics* 33 507 1964
- 18 McMillan J A Landaw S A & Oski F A Iron sufficiency in breast fed infants and the availability of iron from human milk *Pediatrics* 58 686 1976
- 19 Moe P J Normal red blood picture during the first three years of life *Acta Paediatr Scand* 54 1965
- 20 Natvig H & Vællar O B Studies on hemoglobin values in Norway VIII Hemoglobin hematocrit and MCHC values in adult men and women *Acta Med Scand* 187 193 1967
- 21 Sheard C & Sanford A H Photo-electrometer with one stage of amplification *JAMA* 93 1951 1979
- 22 Shojania A M & Hornady G Folate metabolism in newborns and during early infancy II Clearance of folic acid in plasma and excretion of folic acid in urine by newborns *Pediatr Res* 4 477 1970
- 23 Snelling C E The plasma ascorbic acid of infants and children *J Pediatr* 15 8-4 1939
- 24 Sullivan L W Luby A L & Streiff R R Studies of the daily requirement for folic acid in infants and the etiology of folate deficiency in goat's milk megaloblastic anemia *Am J Clin Nutr* 18 311 1966
- 25 Sundal A The norms for height (length) and weight in healthy Norwegian children from birth to 15 years of age *Unn Bergen Arbok Med Series* no 1 3 1956-1957
- 26 Velez H Ghitis J Pradilla A & Vitale J J Calh Harvard nutrition project I Megaloblastic anemia in kwashiorkor *Am J Clin Nutr* 12 54 1963
- 27 Waxman S Folate binding proteins *Br J Haematol* 29 3 1975
- 28 Zuelzer W W & Rutsky J Megaloblastic anemia of infancy *Adv Pediatr* 6 243 1953

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ROLE OF PROLONGED BREAST FEEDING IN INFANT GROWTH

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ABSTRACT Saarinen U. M. and Shimes M. A. (Children's Hospital, University of Helsinki, Finland). Role of prolonged breast feeding in infant growth. *Acta Paediatr Scand* 68: 245, 1979.—The growth of 238 healthy full term infants was followed under a carefully monitored nutritional protocol during the first year of life. The infants were weaned at different ages either to a proprietary infant milk formula or to a home-prepared cow's milk formula. Solid foods were introduced at 3–5 months of age. The 46 infants who were breast fed for a period of at least 6 months were compared to infants weaned prior to one month of age to one of the two milk regimens. In the breast fed infants the weight/weight for height age and skinfold thickness were similar to values in the proprietary formula fed infants but were lower than the corresponding values in the cow's milk fed infants at 6 months of age and subsequently. By using weight for height age as a criterion, no obesity was found among any of the 238 infants and only 17% were considered to be overweight. The results indicate that present recommendations for infant feeding in Finland—including prolonged breast feeding, the use of proprietary milk formulas after weaning, and later introduction of solid foods—prevent over nutrition.

KEY WORDS Breast feeding, infant nutrition, milk, obesity, skinfold thickness.

Obesity is the most common nutritional disorder in developed countries. Since the treatment of established obesity is difficult, more attention has been directed to its prevention.

A high prevalence of excessive weight has been reported in infancy (10–12) with the use of cow's milk based formulas and early introduction of solid foods (7). Presumably such diets predispose to a parent induced calorie excess that is easier to avoid in breast fed infants. In Finland the dietary instructions given by well baby clinics are followed in detail by the majority of parents. This should make it possible to influence calorie intake and avoid overnutrition by appropriate recommendations for infant feeding.

The benefits of breast feeding are currently being re-emphasized for a variety of nutritional, psychological and economic reasons (6). The new national recommendations for infant feeding in Finland given in 1975 stress the use of prolonged breast feeding, delayed

introduction of solid foods, use of water instead of milk or sugar containing fluids for alleviating thirst, and avoidance of additional salt.

In the present study we followed the growth and certain aspects of the nutritional state of breast fed infants in a controlled manner. The infants' adherence to the above mentioned recommendations were monitored in a separate nutrition oriented well baby clinic. After weaning, either a proprietary infant milk formula or a home prepared cow's milk formula was used. The growth of breast fed infants was compared with that of bottle fed ones and with the available growth charts (2).

SUBJECTS

The group of 256 healthy newborn infants had gestational ages between 38 and 47 weeks and a birthweight over 3.0 kg. They were born at the Helsinki University Central Hospital Departments of Obstetrics I and II during the first three months of 1975. All infants whose parents were

two bottle fed control groups were selected as follows (Fig. 1)

Breast milk group Breast milk was the only source of milk in 46 infants until at least the age of 6 months after which they were gradually weaned to proprietary formula or low fat cow's milk (Fig. 1)

Formula group Proprietary formula feeding was started in 47 infants before the age of one month and continued until one year of age. Partial breast feeding was continued to an average of 3 months of age and then discontinued (Fig. 1)

Cow's milk group Cow's milk formula prepared at home was started prior to the age of one month in 9 infants and continued until the age of 11 months after which commercially available low fat cow's milk was used. Partial breast feeding was continued in this group until an average of 7.5 months of age (Fig. 1)

The average daily amounts of milk ingested by the infants were estimated by the mothers and recorded on the history forms under the options of under 100, 100-400, 400-600, 600-800, 800-1000 and over 1000 ml/day. No significant differences were found between the two bottle fed groups by this method.

METHODS

The weight, supine length and skinfold thickness were measured in all infants by the same physician. The weight was taken with the infants naked on a single calibrated scale. Supine length was measured with the infant in a trestle position on a special table fitted with a sliding footboard after the baby had been straightened, the legs stretched, the toes turned upwards, and the sliding board then brought into contact with the heels. The thicknesses of the triceps and subscapular skinfolds were measured at the ages of 6, 9 and 12 months with a Harpenden skinfold caliper using the technique of Tanner & Whitehouse (15). A mean value of three consecutive measurements was used.

Classification of the infants

The individual values for weight and length at different ages were plotted on magnified growth charts (7) and expressed as standard deviation scores. In this manner we were able to eliminate the error that would arise if an infant was not seen on the exact date of the age category. This deviation in age was less than 4 days at 2 weeks of age, less than 7 days up to and including 4 months of age and less than 2 weeks at the later ages.

After the statistical analyses the final results were converted back to cm and kg.

Definition of breast overweight and underweight

The classification is based on the method recommended by Smith & Wilkinson (11). The weight for height age was obtained by determining the age at which the infant's actual height falls on the 50th percentile (7) and then finding the mean weight corresponding to that age. The actual weight was compared to this mean weight and expressed as a standard deviation score. Obesity was defined as

weight for height age above $+2$ S.D. and overweight between $+1$ S.D. and $+2$ S.D. Similarly thinness was defined as a weight for height age below -2 S.D. and underweight between -1 S.D. and -2 S.D.

Statistical analyses

In order to select the appropriate statistical methods we determined whether measurements followed a Gaussian distribution by using a chi square test. All distributions were normal for weight and supine length though skewed distributions for weight have been reported elsewhere (14, 16). Thus it was appropriate to use Student's *t* test in these statistical analyses. For skinfold thickness values the chi square test gave skewed distributions only in certain instances in the formula group, e.g. the subscapular skinfold at 9 months and the triceps skinfold at 12 months of age. Because significant differences were found only between the breast milk group and the cow's milk group for data with normal distributions the Student's *t* test was used for these comparisons. Analysis of variance which does not require a Gaussian distribution was used in addition.

RESULTS

The values for body length in the breast milk group and the two bottle fed groups are shown in Table 2. The breast milk group differed significantly from the cow's milk group only if boys were separately analysed at 9 months of age ($p < 0.02$) and at 12 months of age ($p < 0.05$). The infants in all three groups were taller than the reference values (2) during the latter half of infancy.

The body weight of the three milk groups is shown in Table 2. Infants of the breast milk group weighed significantly less than infants of the cow's milk group. Among the girls differences were found at ages 4 ($p < 0.05$), 6 ($p < 0.005$), 9 ($p < 0.005$) and 12 months ($p < 0.01$) and in the boys at ages 6 ($p < 0.05$), 9 ($p < 0.005$) and 12 months ($p < 0.01$). The girls in the formula group weighed less than the girls in the cow's milk group at ages 4, 6, 9 and 12 months ($p < 0.01$ or $p < 0.02$) but there were no corresponding significant differences among the boys. The weight gains in the breast milk group and the formula group were similar in both sexes.

The weight differences between the three milk groups during the first year of life are expressed in grams in Fig. 2 where the mean

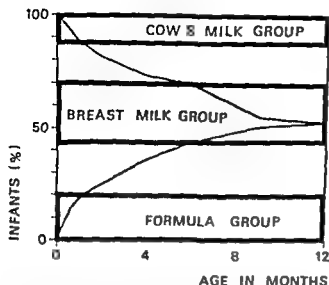


Fig. 1 Milk feeding of 238 infants. The darker shaded area shows the proportion of infants entirely on breast milk. Partial breast feeding continued after the initiation of one of the two other milks, as indicated by the lighter zones on both sides of the breast milk area. The borders indicate how the breast milk group, cow's milk group and formula group were formed.

willing to join the program were included and were examined at 2 weeks, 1, 2, 4, 6, 9 and 12 months of age. The final series consisted of 238 infants, 126 boys and 112 girls, since 18 of the 256 infants dropped out of the study during the course of the year.

Solid foods and supplementation

Solid foods were introduced according to a strict protocol and monitored by history and careful instructions at each visit as follows: cooked vegetables and fruit at 3–5 months, cereals at 5 months, meat and eggs at 6 months and a normal mixed diet at 9 months of age. This represents a later introduction of solid foods than has been customary in Finland and most other developed countries in recent years. We also stressed the use of water instead of milk for thirst. No sugar was added to the water except during acute febrile illnesses, and no additional salt was used before 8 months of age. No supplemental iron was given to

the infants fed on breast milk or cow's milk but the proprietary formula was supplemented with 11 mg of elemental iron per liter. At 2 weeks of age daily vitamin supplementation was started: vitamin D 1000 IU, vitamin A 1500 IU and vitamin C 20 mg.

Milk feeding

All mothers were advised to use breast milk initially as the only source of milk. This recommendation resulted in breast milk being used exclusively by 138 infants until 6 months, 92 infants to 4 months, 46 infants to 6 months and 8 infants to 9 months of age (Fig. 1). This incidence of breast feeding was much higher than what was recently estimated to be customary among the general infant population in Finland, e.g. a 9% incidence of breast feeding at 6 months of age in the Helsinki area in 1966 (8).

Infants taking part in the study were identified by sequential numbers at the time of birth. The infants given an odd number were later weaned to proprietary milk formula (Table 1) and those with an even number were weaned to a home prepared cow's milk formula. For infants under 6 months of age the latter formula was prepared as follows: 600 ml of commercially available pasteurized homogenized dairy milk (3.9% fat) and 400 ml of water were mixed and brought to boiling point after which 50 g lactose was added (Table 1). For infants over 6 months of age commercially available low fat (1.5%) milk was used as such (Table 1). Partial breast feeding was continued as indicated in Fig. 1. The daily amount of milk recommended was one fifth of the infant's weight up to 1 liter.

The analysis of the data revealed that those infants belonging to the groups with odd numbers were generally weaned somewhat earlier than the infants with even numbers (Fig. 1). This difference was thought to be purely coincidental. However, one might speculate that the difference could be partly influenced by a certain degree of unwillingness on the part of the mothers to start their baby on home prepared cow's milk formula which perhaps was regarded as a somewhat old-fashioned and inconvenient regimen. This attitude might have predisposed to prolong breast feeding.

Study groups

From the total of 238 infants, one breast fed group and

Table 1 Composition of milks used in the study

In the proprietary formula also vitamins and minerals were added which are not shown in the table

	Breast milk	Proprietary formula	Home prepared cow's milk formula	Commercial low fat cow's milk
Energy (kcal/l)	680	675	670	550
Lactose (g/l)	70	72	80	45
Proteins (g/l)	11	15	70	34
Casein (%)	40	40	80	80
Whey (%)	60	60	20	20
Fats (g/l)	36	35	23	25
Saturated fatty acids (%)	~50	36	73	73
Unsaturated fatty acids (%)	~50	64	27	27

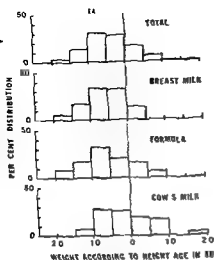


Fig. 3. Distribution and mean of weight for height age scores in the entire series and in the three milk groups. The weight for height age value was determined for each infant individually at one year of age and expressed as a standard deviation score. Entire series (total) $n=738$. Breast milk group $n=66$. Formula group $n=47$. Cow's milk group $n=9$.

age (Student's t test $p<0.001$, analysis of variance F test $p<0.01$). The thicknesses of the triceps and subscapular skinfolds are shown separately for boys and girls (Fig. 4) and indicate the same differences in each sex. The girls had slightly thicker subscapular skinfolds than the boys, although this difference was of statistical significance only at 6 months of age (analysis of variance F test $p<0.05$).

DISCUSSION

In this investigation we compared the growth of infants who were breast fed for a period of at least 6 months with infants fed on two other milk regimens. All infants followed the same regimen for solid foods.

In early infancy there were only small differences among the groups of infants on the three regimens. The breast fed infants gained slightly more weight than those fed cow's milk based formulas, but not to a statistically significant degree. During the latter half of infancy the breast milk group was found to be lowest in body weight.

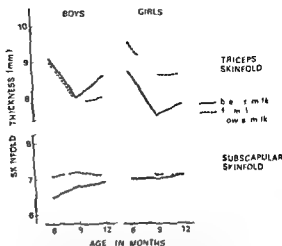


Fig. 4. Thickness of the triceps and subscapular skinfolds at the ages of 6, 9 and 12 months. The lines indicate the mean values of the study groups shown for boys and girls separately.

The weight gain of infants fed proprietary formula was similar to that of the breast fed infants. This finding contradicts the commonly held notion that bottle feeding always results in a greater weight gain than breast feeding (5) and is in accord with earlier observations that artificially fed infants can gain weight at approximately the same rate as breast fed infants (9).

None of the infants were malnourished as noted during frequent physical examinations and as shown by a normal growth in length. In fact the infants were somewhat taller on the average than the reference norms for Finnish babies obtained 15 to 20 years ago (2).

The infants of the cow's milk group had the highest weight, weight for height age and skinfold thickness. Because no differences were noted in the volumes of milk consumed and since the energy content of the commercial low fat cow's milk used after 6 months of age was lower than that of breast milk or the proprietary formula, the differences in growth might be due to the composition of these milks and especially in respect to the protein and fat composition.

There is a widespread tendency to introduce non-milk foods at an early age, although no

Table 2 *Supine length in cm (mean \pm S D left) and weight in kg (mean \pm S D right) of the breast fed infant group and the two bottle fed control groups*

Breast milk				
Age (mo)	Boys (n=33)		Girls (n=23)	
0	50.7 \pm 1.6	3.7 \pm 0.4	50.1 \pm 1.6	3.5 \pm 0.3
0.5	53.7 \pm 2.1	3.8 \pm 0.5	52.8 \pm 1.7	3.6 \pm 0.3
1	55.9 \pm 2.2	4.4 \pm 0.6	55.0 \pm 1.5	4.1 \pm 0.3
2	59.2 \pm 2.2	5.4 \pm 0.6	58.3 \pm 1.9	5.0 \pm 0.3
4	65.3 \pm 2.1	6.9 \pm 0.9	63.7 \pm 1.7	6.5 \pm 0.4
6	68.8 \pm 2.4	7.8 \pm 0.9	67.7 \pm 2.0	7.4 \pm 0.6
9	73.4 \pm 2.3	9.0 \pm 1.0	72.7 \pm 1.8	8.5 \pm 0.6
12	77.5 \pm 2.4	10.0 \pm 0.9	76.5 \pm 2.0	9.5 \pm 0.8

Cow's milk				
Age (mo)	Boys (n=13)		Girls (n=16)	
0	50.4 \pm 1.3	3.8 \pm 0.3	49.8 \pm 2.1	3.5 \pm 0.4
0.5	53.4 \pm 1.2	3.7 \pm 0.4	52.5 \pm 1.6	3.6 \pm 0.4
1	55.3 \pm 1.3	4.2 \pm 0.5	54.6 \pm 1.7	4.0 \pm 0.5
2	59.0 \pm 1.6	5.2 \pm 0.5	58.2 \pm 1.7	4.9 \pm 0.5
4	65.3 \pm 1.6	7.1 \pm 0.9	64.3 \pm 1.3	6.8 \pm 0.4
6	69.3 \pm 2.0	8.4 \pm 0.9	68.3 \pm 1.6	7.9 \pm 0.4
9	75.1 \pm 1.8	10.0 \pm 1.0	73.5 \pm 1.7	9.2 \pm 0.7
12	79.2 \pm 2.3	11.0 \pm 1.1	77.2 \pm 2.2	10.3 \pm 0.9

Formula				
Age (mo)	Boys (n=29)		Girls (n=18)	
0	51.4 \pm 1.7	3.8 \pm 0.4	49.5 \pm 1.8	3.4 \pm 0.3
0.5	53.6 \pm 2.0	3.8 \pm 0.4	52.5 \pm 1.7	3.5 \pm 0.4
1	56.0 \pm 2.0	4.3 \pm 0.4	54.6 \pm 1.6	3.9 \pm 0.3
2	59.4 \pm 2.4	5.4 \pm 0.6	57.8 \pm 1.5	4.7 \pm 0.4
4	65.6 \pm 2.3	7.2 \pm 0.7	63.4 \pm 1.5	6.3 \pm 0.6
6	69.6 \pm 2.2	8.3 \pm 0.8	67.9 \pm 1.9	7.3 \pm 0.8
9	74.4 \pm 2.4	9.4 \pm 0.8	72.1 \pm 1.8	8.5 \pm 0.9
12	78.1 \pm 2.5	10.3 \pm 0.9	76.6 \pm 2.3	9.4 \pm 1.0

weight of the breast milk group represents the baseline. In early infancy the breast fed infants gained slightly more weight than the bottle fed infants although these differences did not reach statistical significance. After 4 months of age there was a gradual change in the opposite direction.

The mean weight for height age and its distribution at one year of age is shown in Fig 3 for each of the three milk groups as compared to the entire series of 238 infants using the distribution of individual standard deviation

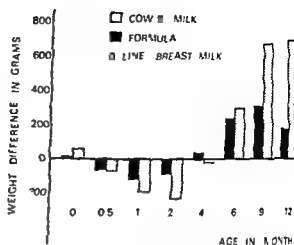


Fig. 2 Development of weight differences between breast milk group and the two bottle fed control groups during the first year of life. Values of the breast milk group are used as reference standard and represent zero line.

scores. The mean weight for height age of the entire series was -0.43 S D. In the breast milk group the mean score was -0.69 S D and the formula group -0.62 S D. The mean value of -0.09 S D of the cow's milk group is higher than those of the two other groups ($p < 0.001$) and this difference was significant using the nonparametric U test of Mc Nemar & Whitney.

At one year of age none of the infants in breast milk or formula groups were obese weight but 1 out of 29 infants in the cow's milk group was. In the entire series no infant was found to be obese and 4 out of 238 were overweight. Of these 4 infants 2 had been overweight to cow's milk and 2 to formula prior to months of age.

The proportion of underweight infants according to the criteria used was 22% in the breast milk group, 26% in the formula group and 6.9% in the cow's milk group at one year of age. None was found to be thin.

The infants of the cow's milk group were found to have the most subcutaneous fat measurements of the triceps and subscapular skinfolds (Fig. 4). However the difference between the cow's milk group and the breast milk group was only statistically significant in the triceps skinfold thickness at 12 months.

GROWTH HORMONE RESPONSE TO PROSTAGLANDIN E

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ABSTRACT Hamilton W and Hussein D M (University Department of Child Health Royal Hospital for Sick Children, Yorkhill, Glasgow). Growth hormone response to prostaglandin E. *Acta Paediatr Scand* 68 251 1979.—C AMP causes release of growth hormone from the pituitary. Prostaglandin increases pituitary adenylate cyclase and C AMP and therefore acts like a growth hormone-releasing hormone. Using PGE_2 (30 μg per kilo body weight) given intravenously we have demonstrated that in no case was there a growth hormone response to insulin hypoglycaemia when PGE_2 failed to evoke a response. However in 6 of 11 patients unresponsive to insulin hypoglycaemia a significant rise in growth hormone was obtained from PGE_2 . We argue that in these 6 patients hypothalamic unresponsiveness to hypoglycaemia must be operative whereas PGE_2 acting directly on pituitary level is likely to have caused the release of preformed growth hormone from the pituitary. Administration of PGE_2 does not cause hypoglycaemia but rather a slight rise in the plasma glucose level. Thus the risk of brain damage which is inherent in the insulin hypoglycaemia test is avoided.

KEY WORDS Growth hormone, insulin hypoglycaemia test, prostaglandin, stimulation.

A number of tests have been designed to evaluate the pituitary reserve of growth hormone. These have been evaluated by Fraser (2). Good screening tests detect all patients who are likely to have inadequate growth hormone reserve while the ideal definitive test detects only patients who are growth hormone deficient. However it would appear that such tests as insulin hypoglycaemia, arginine infusion and oral L dopa, all highly satisfactory according to the above criterion for a definitive test, utilize hypothalamic mechanisms to increase circulation of growth hormone levels. Hypoglycaemia acting through glucoprivation at the lateral hypothalamus results in release of growth hormone releasing hormone (GHRH) which however still awaits identification and characterization. L Dopa is converted in the brain to dopamine and via dopaminergic receptors at the lateral border of the ventromedial nucleus stimulates the release of GHRH. Arginine and glycine probably act as neuroinhibitors of cells in the median eminence decreasing the release of growth hormone release inhibitory hormone (GHRH or

somatostatin). These amino acid tests may therefore specifically detect growth hormone deficiency due to excessive inhibition of growth hormone release from the pituitary (1). Such a cause of short stature has not yet been demonstrated although the possibility has been suggested (1). If however a test agent were available to stimulate the pituitary to secrete growth hormone then separation of hypothalamic and pituitary causes for growth hormone deficiency would be possible. Additionally at present it is not clear if a lack of response to the insulin hypoglycaemia test indicates that hypothalamo-pituitary stimulation is defective or that the pituitary cells lack adequate growth hormone synthetic ability. The therapeutic implications of such a test substance would be its possible use as a stimulating agent to induce pituitary output of preformed growth hormone if only hypothalamic mechanism were defective.

Prostaglandins are known to increase growth hormone secretion (3, 4). It is assumed

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evidence is available to indicate that this achieves a better nutritional state. On the contrary it appears to result in excessive weight gain (13). In the 1950s, when our reference norms (2) were obtained the use of home prepared cow's milk formulas was common in this country. Our present dietary regimen differed from the customary practice in the 1940s and 1960s in several ways that might influence weight gain, such as postponement of the introduction of solid foods, use of sugar free water for thirst and avoidance of added salt. Our data, however, do not offer sufficient evidence in support of the role of the above mentioned factors in infant growth, since no essential differences were found between our cow's milk group and the reference norms in the weight gain, weight for height, age or proportion of overweight infants. Studies on 1684 infants in Helsinki area in 1966 showed that 11% were obese and 5.4% were overweight at one year of age by using the same criteria for obesity and overweight as used in this study (8), compared to 3.4% of overweight infants in our cow's milk group.

Successful breast feeding for 6 months or more can be achieved by mothers if they are actively encouraged and when the infants are closely followed by trained personnel. Although a number of identifiable factors are involved in the pathogenesis of overnutrition we feel that prolonged breast feeding is a natural way to promote normal growth and possibly decrease the risk of later obesity (1, 3, 4).

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REFERENCES

- 1 Asher P. Fat babies and fat children: The prognosis of obesity in the very young. *Arch Dis Child* 41: 672, 1966.
- 2 Backstrom L & Kantero M L. Cross sectional

studies of height and weight in Finnish children aged from birth to 20 years. *Acta Paediatr Scand* Suppl. 220: 9, 1971.

- 3 Charnov E, Goodman H, C M, Brade M, Linn B & Pratt H. Childhood antecedents of adult obesity: Do chubby infants become obese adults? *N Engl J Med* 195: 8, 1976.
- 4 Lid L L. Follow up study of physical growth of children who had excessive weight gain in the first six months of life. *Br Med J* 11: 74, 1970.
- 5 Fomon S J & May C D. Metabolic studies of normal full term infants fed pasteurized human milk. *Pediatrics* 22: 101, 1958.
- 6 IPA Seminar Montreux Aug 15-19, 1975. Recommendations to action programmes to encourage breast feeding. *Acta Paediatr Scand* 65: 775, 1976.
- 7 Jackson R L, Westerfeld R, Flynn M A, Kimball E R & Lewis R B. Growth of well born American infants fed human and cow's milk. *Pediatrics* 31: 642, 1964.
- 8 Kantero R L. Unpublished observations, 1966.
- 9 Puva S I. Pattern of growth of selected groups of breast fed infants in Iowa city. *Pediatrics* 11: 8, 1943.
- 10 Shukla A, Forsyth H A, Anderson C M & Marwah S M. Infantile overnutrition in the first year of life: A field study in Dudley, Worcestershire. *Br Med J* 16: 507, 1972.
- 11 Smith D W. Growth and its disorders: Basis and standards: approach and classification: growth deficiency disorders: growth excess disorders: obesity. In A J Schaffer & M Markowitz (eds.) *Major problems in clinical pediatrics*. W B Saunders & Company, Philadelphia, 1977, vol. XX, p. 2.
- 12 Sveger T, Lindberg B, Weibull B & Olsson L L. Nutrition, overnutrition and obesity in the first year of life in Malmö, Sweden. *Acta Paediatr Scand* 64: 635, 1975.
- 13 Taitz L S. Infantile overnutrition among artificially fed infants in the Sheffield region. *Br Med J* 1: 319, 1971.
- 14 Tanner J M. Physical growth and development. In J O Forfar & G C Arneil (eds.) *Textbook of Pediatrics*. Churchill Livingstone, Edinburgh and London, 1973.
- 15 Tanner J M & Whitehouse J H. Revised standards for triceps and subscapular skinfolds in British children. *Arch Dis Child* 50: 142, 1975.
- 16 Tanner J M, Whitehouse J H & Takarashi M. Standards from birth to maturity for height, weight, height velocity and weight velocity: British children, 1965. *Arch Dis Child* 41: 613, 1966.

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Table 2 *Insulin hypoglycaemia and prostaglandin E tests in ten children with short stature (both tests positive)*

Patient No /Sex	Age (years)	Plasma growth hormone ($\mu\text{g/l}$)						Plasma glucose in response to PGE ₂ (mmol/l)		
		IHT			PGE ₂			0	30	60
		0	Max	Time	0	30	60			
13 F	7.4	4.5	30.0	60	2.25	21.0	25.8	11	2.4	3.3
14 M	8.7	7	30.0	90	0.9	1.1	11.4	1.7	1	3.3
15 M	9.7	0.9	0.6	60	1.5	7.5	15.5	1.6	2.6	2.6
16 F	10.0	13.5	30.0	5	10.5	8.5	11.1	2.0	2.4	2.1
17 M	10.0	4.0	21.2	0	16	21.8	14.7	1.1	1.4	2.0
18 M	11.8	14.7	23.5	90	13	13.5	13.0	1.6	1.5	2.3
19 M	13.3	3.3	23.9	30	4.4	11.4	30.0	2.1	2.4	2.3
0 M	14.7	1.5	30.0	90	14.3	11.0	30.0	1.7	1.9	2.1
1 M	16.7	1.5	21.3	60	1.1	1	15.8	3	8	1.8
2 M	16.9	30.0	30.0	60	11.4	30.0	30.0	1.5	2.3	2.1

therapy. It will be further noted that in these 12 patients PGE₂ did not provoke a significant increase in the circulating levels of growth hormone and neither did it cause hypoglycaemia but rather a slight rise in plasma glucose levels.

In Table 2 are results from a further 10 patients in whom both tests gave an adequate growth hormone response. Here again PGE₂ did not provoke hypoglycaemia but in 5 out of 10 there was an increase (around 2 fold) in the blood glucose levels.

In Table 3 are the results from 6 patients in whom hypoglycaemia failed to provoke a growth hormone response while the response

to PGE₂ indicated adequate growth hormone reserve. Two of these patients are now receiving growth hormone therapy. Again in this group PGE₂ induced a slight increase in blood glucose levels.

In Table 4 are the effects of PGE₂ on the plasma levels of TSH, LH and FSH in those patients for whom sufficient plasma was available. Only TSH levels showed an increase in 8 of 19 patients. LH and FSH levels were unaffected in the 10 samples assayed.

The overt clinical response to PGE₂ varied from mild to moderate but immediate nausea, vomiting, abdominal discomfort, headache and a desire to micturate and defaecate were

Table 3 *Insulin hypoglycaemia and prostaglandin E tests in six children with short stature (IHT negative, PGE₂ positive)*

Patient No /Sex	Age (years)	Plasma growth hormone ($\mu\text{g/l}$)						Plasma glucose levels in response to PGE ₂ (mmol/l)		
		IHT			PGE			0	30	60
		0	Max	Time	0	30	60			
3 F	6.7	6.5	4.9	60	2.5	4.4	11.3	2.0	2.3	2.7
4 F	10.1	5.6	5.3	5	3	1.1	11.0	1.2	2.1	2.1
5 M	10.8	0.1	8.1	30	2.3	7.7	17.9	3.3	2.9	3.4
6 M	11.6	0.1	1.8	1.0	1.8	30.0	3.6	2.4	3.1	3.4
7 M	16.1	6.9	9	30	2.3	9.3	18.3	2.6	3.3	3.4
28 M	17.3	2.7	5.1	1.0	5.1	13.4	13.2	2.1	2.2	3.4

Patients now receiving growth hormone as therapy

Table 1 Insulin hypoglycaemia and prostaglandin E_2 tests in twelve children with short stature (both tests negative)

Patient No/Sex	Age (years)	Plasma growth hormone ($\mu\text{g/l}$)						Plasma glucose levels in response to PGE ₂ (mmol/l)		
		IHT			PGE ₂					
		0	Max	Time	0	20	60	0	20	60
1 F	7.5	4.8	3.4	120	3.4	2.8	5.3	3.4	4.5	4.8
2 M*	9.2	1.5	2.1	10	0.3	0.6	1.5	3.6	4.1	5.1
3 M	9.6	2.1	6.8	120	6.8	3.8	1.2	4.3	4.8	5.1
4 M	9.7	0.1	4.6	60	0.9	0.5	1.3	2.9	4.8	5.5
5 M	9.9	0.8	4.1	90	0.9	2.3	5.4	3.2	4.4	5.2
6 M	10.1	0.1	4.7	60	0.9	1.1	0.5	5.2	5.1	5.6
7 M*	10.5	0.8	0.3	120	2.5	2.4	0.8	3.1	5.0	4.8
8 F	10.9	0.3	0.9	120	0.9	1.1	0.5	5.2	5.6	6.1
9 M	11.3	3.6	5.3	60	3.5	2.0	0.3	3.3	4.0	4.6
10 M	11.9	0.1	6.3	60	5.9	5.7	7.0	4.0	4.6	5.2
11 M	14.1	0.2	2.8	60	0.3	1.5	3.0	3.1	5.1	6.8
12 M	15.0	0.3	0.4	60	0.3	1.4	0.1	4.2	5.4	5.8

* Patients now receiving growth hormone as therapy

by several workers that its action is through an increase in intracellular 3',5' cyclic AMP at pituitary level (5). This has led us to explore the value of prostaglandin (PGE₂) as a test substance for growth hormone reserve in children with short stature.

MATERIALS AND METHODS

The patients investigated were 21 boys and 7 girls whose ages ranged from 6.6 to 17.3 years. All were of short stature and by virtue of their anthropometric data and their lack of other organic growth retarding disease required endocrine investigation. The investigations were carried out by permission of fully informed parents.

The insulin hypoglycaemia test (IHT) was performed after an overnight fast. A slow intravenous infusion (0.45% saline) was established and at zero time soluble insulin (0.1 units per kilo body weight) was given intravenously. In the absence of clinical hypoglycaemic features at 30 min a second dose of insulin (0.1 units per kilo body weight) was given. Lithium heparinized blood samples were taken at 0, 5, 10, 20, 30, 60, 90 and 120 min. At 120 min PGE₂ (30 μg per kilo body weight) was given intravenously (3) as an undiluted bolus. Further blood samples were taken at 140 and 180 min using the 120 min sample for the basal level for the PGE₂ response. PGE₂ (Upjohn Ltd, Crawley, England) was used. Growth hormone was assayed by a double antibody radioimmunoassay technique (CIS kit, Eurotype Services Ltd, Finchley, N 12).

Plasma glucose levels were assayed by a Bechman analyzer method. Retrospectively when sufficient plasma remained, LH, FSH and TSH were assayed on the post

PGE₂ samples. Double antibody radioimmunoassay methods were used (CIS kit, Eurotype Services Ltd, Finchley, N 12) for these assays.

RESULTS

To interpret our data we have considered that absolute deficiency of growth hormone exists when the plasma growth hormone levels do not rise above 7 $\mu\text{g/l}$ following adequate hypoglycaemia. Partial deficiency is represented by plasma levels between 7 and 10 $\mu\text{g/l}$ while values greater than 10 $\mu\text{g/l}$ are regarded as representing normal growth hormone reserve. To assess the significance of the pituitary responsiveness to PGE₂ we have used these same plasma levels of growth hormone. Adequate hypoglycaemia is represented by plasma glucose levels not exceeding 1 mmol/l. Plasma glucose levels following insulin administration are not quoted since satisfactory hypoglycaemia was ensured in all patients.

In Table 1 are the levels of plasma growth hormone in response to insulin and PGE₂ in 12 patients unresponsive to the IHT. In each case only the zero and the peak values are given with the corresponding time interval for the peak value. The patients marked * have subsequently been started on growth hormone

response can be obtained in the face of a partial response from either alone. The intra venous glycine test has been reported to give an adequate growth hormone response in 3 of 50 patients in whom the IHT was negative. In 4 patients of the same series the glycine test was negative while the IHT was adequate (1). These authors suggest that glycine acts by inhibiting somatostatin activity at the pituitary level and not by stimulating GH RH. The PGE_2 test would appear to supercede these amino acid tests since in our series there was no case responsive to the IHT and negative to PGE_2 . Here we argue that direct pituitary stimulation is a theoretical possibility accounting for our findings in those patients who did not respond to insulin hypoglycaemia but who did respond to PGE_2 .

Hypothalamic extracts stimulate adenylate cyclase activity and C AMP concentrations in the anterior pituitary (8). C AMP causes release of growth hormone from the pituitary (5). PGE_2 also increases pituitary adenylate cyclase and C AMP (5). Thus if PGE_2 fails to provoke an increased level of circulating growth hormone the inference is that there has been failure of growth hormone synthesis by the pituitary cells. This distinction between failure of synthesis and failure of release of growth hormone is not made by utilizing a test which is mediated via the hypothalamus since it is not possible to determine whether or not failure to increase growth hormone from a hypothalamic mediated response indicates failure of hypothalamic responsiveness or lack of pituitary growth hormone reserve. The PGE_2 test may therefore identify patients whose short stature is due to failure of adequate growth hormone synthesis.

It will be noted in our regime that IHT and the PGE_2 test have been performed at the same time. Other workers have suggested that at least a 24 hour period should elapse between two pituitary stimulation tests thereby suggesting the possibility of pituitary exhaustion. We do not think that this is relevant and our data would support that view. Additionally,

the PGE_2 test may be performed without an overnight fast and at any time of the day. It is therefore particularly useful for patients in whom there is a history of convulsions or of spontaneous hypoglycaemic attacks. In such patients failure to increase growth hormone levels to PGE_2 stimulation alerts the investigator to the need for consummate caution if an IHT is undertaken.

TSH and ACTH are also released by prostaglandins but not LH nor prolactin (5). Our data show that PGE_2 stimulation does give a rise in plasma TSH levels (8 of 19) but no response was noted in LH or FSH levels (10 of 10). Our selection for the timing of plasma sampling in the PGE_2 test was partly to conform to the recommended timing for the LRH/TRH test. These times (0, 20, 60 min) seemed appropriate because the PGE_2 test gives immediate predictable clinical responses which continue throughout the first twenty minutes post injection followed by a slow reduction in the intensity of the reactions until sleep ensues by sixty minutes.

The PGE_2 test as described was unpleasant for all the children but in 6 the only side effect was transient pain at the injection site. There are as far as we judge no risks and the test need never be repeated because of failure to achieve a clinical response. PGE_2 does not cause hypoglycaemia so that the patient is at no risk from hypoglycaemic cerebral damage nor anoxia following convulsions.

The PGE_2 test at present does not replace all other tests because growth hormone therapy is still the only treatment when both the IHT and PGE_2 test are negative. It is therefore still necessary to perform the IHT if the PGE_2 test is positive.

It might be argued that the growth hormone response to PGE_2 was part of a systemic reaction and not specifically a pituitary response. Notwithstanding this possibility intravenous prostaglandin is worthy of further investigation as a test procedure to separate hypothalamic from pituitary causes of growth hormone deficiency.

Table 4 Plasma levels of TSH, LH and FSH in response to intravenous prostaglandin E

UD = Undetected

Patient No./Sex	Age (years)	Plasma TSH (mU/l)			Plasma LH (U/l)			Plasma FSH (U/l)		
		0	20	60	0	20	60	0	0	60
23 F	6.7	6.0	5.5	4.4						
13 F	7.4	4.0	4	4	1.8	1.8	1.8	0.9	0.9	0.9
14 M	8.7	4.9	16.8	10.9						
2 M	9.2	2.2	6.7	2.1	3.1	3.1	3.1	0.7	0.7	0.7
3 M	9.6	2.1	2.1	2.1	3.1	3.1	3.1	0.7	0.7	0.7
4 M	9.7	UD	5.6	2.9	3.1	3.1	3.1	0.7	0.7	0.7
16 F	10.0	4.4	35.3	20.2						
6 M	10.1	2.6	2.1	2.1	3.1	3.1	3.1	1.0	1.0	1.0
7 M	10.5	5.3	8.2	5.8	3.1	3.1	3.1	0.7	0.7	0.7
25 M	10.8	6.9	5.7	6.0	3.1	3.1	3.1	0.7	0.7	0.7
8 F	10.9	2.1	11.7	2.1	3.1	3.1	3.1	0.7	0.7	0.7
26 M	11.6	2.9	3.4	2.9						
18 M	11.8	2.5	9.8	5.2	2.0	1.3	1.2	1.9	1.9	1.9
19 M	13.3	3.1	2.7							
20 M	14.7	1.6	1.5	1.5						
12 M	15.0	5.3	4.7	4.7	3.1	3.1	3.1	0.7	0.7	0.7
27 M	16.1	2.9	2.9	2.9						
21 M	16.2	1.5	1.5	1.5						
22 M	16.9	2.5	2.4	1.9						

noted. All patients were significantly drowsy after the test and slept for up to 2 hours.

DISCUSSION

It is generally accepted that the secretion of growth hormone occurs physiologically in response to sleep and to hypoglycaemia. The insulin hypoglycaemia test (IHT) utilizes physiological hormone reflexes to induce growth hormone release from the pituitary. It is however possible that altered hypothalamic responsiveness to glucoprivation could result in lack of growth hormone releasing hormone (GHRH) or in a disproportionate amount of somatostatin thus resulting in a peripheral lack of growth hormone even although the pituitary reserve of growth hormone were adequate. Were such a situation to obtain it should be possible to find a negative growth hormone response to hypoglycaemia but an adequate response following a test which directly stimulates the pituitary to release its growth hormone. In theory therefore short stature attributable to lack of growth hormone could result from a lesion of the hypothalamus or from a lack of endogenous

growth hormone in the pituitary. At present there is no real clinical or biochemical separation of these possible aetiologies and treatment of both (were they to exist) would presently be by growth hormone administration.

If however short stature were due to a hypothalamic abnormality where pituitary growth hormone reserve was of itself adequate then rational treatment would be the administration of a drug known to stimulate directly the pituitary to release its growth hormone.

The data which we present here demonstrate that in those children tested there were some with the traditionally accepted growth hormone lack in response to hypoglycaemia but who showed a normal growth hormone response to PGE₁. Conversely in this series the PGE₁ test did not indicate lack of growth hormone reserve in any patients when the IHT gave an adequate response.

Many authorities believe that a child should be demonstrated to have growth hormone deficiency on two different tests before administering growth hormone as therapy. There is published evidence (7) that with the combined L-arginine and L-dopa test an adequate

SERUM CONCENTRATIONS OF THYROTROPIN THYROID HORMONES AND THYROID HORMONE BINDING PROTEINS DURING ACUTE AND RECOVERY STAGES OF IDIOPATHIC RESPIRATORY DISTRESS SYNDROME

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ABSTRACT Jacobsen B B Peitersen B and Hummer L (University Clinic of Paediatrics Children's Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Serum concentrations of thyrotropin thyroid hormones and thyroid hormone-binding proteins during acute and recovery stages of idiopathic respiratory distress syndrome *Acta Paediatr Scand* 68 257 1979.—A total number of 77 premature infants with idiopathic respiratory distress syndrome (IRDS) and 52 healthy controls with comparable gestational age and body weights were studied during the first month of life. In infants with IRDS a reduced thyrotropin (TSH) response to birth was suggested as serum TSH was lower in IRDS patients than in controls during the first two days of life. Low serum concentrations of thyroid hormones were found in the acute stage of IRDS reaching minimal values by day 3-5. After that period an increase in thyroid hormone levels occurred. The serum T_3 increased to the level of healthy prematures by day 6-10 whereas the serum T_4 increased to normal levels by day 21-30. Serum concentrations of thyroxine-binding globulin (TBG) were significantly lower in IRDS patients than in healthy controls; a gradual increase to normal levels occurred during recovery. Serum prealbumin (TBPA) levels in IRDS infants increased rapidly after birth and exceeded levels of healthy infants. Serum albumin values were not significantly different in the two groups of infants. The serum T_4 /TBG ratios were low during recovery from IRDS.

KEY WORDS Thyrotropin thyroid hormones thyroxine binding globulin prealbumin albumin idiopathic respiratory distress syndrome

In 1974 studies by Redding & Pereira (24) showed a significantly decreased total serum thyroxine concentration in infants with idiopathic respiratory distress syndrome (IRDS). In 1976 Cuestas et al (5) reported that cord serum triiodothyronine also was lower in infants with IRDS than in those without. These findings have been confirmed by others (1, 17). The pathogenetic significance of the low thyroid hormone levels is obscure but studies in fetal and adult animals have indicated an important influence of the thyroid hormones upon lung growth and production of the pulmonary surfactant factor (8, 23, 29). Furthermore a relatively high incidence of IRDS in neonatal hypothyroidism in man has been reported (28).

The mechanisms that determine diminished thyroid hormone levels in IRDS infants are unknown but a decreased activity of the thyroid

gland has been suggested (5, 6, 17, 24). Recently we reported preliminary results indicating low serum thyroxine binding globulin concentration in IRDS infants (13, 15). The changes in serum concentrations of thyroid hormones following recovery from IRDS have not been fully elucidated (1, 18) and no studies of the thyroid hormone binding protein levels have been reported. In the present study therefore we determined serum concentrations of thyroid hormones and hormone binding proteins in infants during the acute and recovery stages of IRDS and compared the results with those obtained in healthy prematures.

MATERIALS AND METHODS

A total number of 27 premature infants with IRDS was studied. The diagnosis of IRDS was based upon clinical biochemical and radiological data (16, 26). The treatment was carried out in accordance with previous report (16).

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REFERENCES

- 1 Florea I, Iopu M, Simionescu L, Dinulescu E & Juvine E. Glycine intravenous load for diagnosis of growth hormone deficiency. *Clin Endocrinol* 5: 283, 1976.
- 2 Fraser D S. A review of growth hormone stimulation tests in children. *Pediatrics* 53: 979, 1974.
- 3 Hertelendy F, Todd H, Ehrhart K & Blute R. Studies on growth hormone secretion. IV. In vivo effects of prostaglandin E1. *Prostaglandins* 7: 79, 1972.
- 4 Ito H, Momse G, Katayama T, Takagishi H, Ito I, Nakayama H & Takei Y. Effect of prostaglandin on the secretion of human growth hormone. *J Clin Endocrinol Metab* 37: 857, 1971.
- 5 MacLeod R & Lehmeyer J E. Release of pituitary growth hormone by prostaglandins and dibutyryl adenosine cyclic 3',5' monophosphate in the absence of protein synthesis. *Proc Natl Acad Sci* 67: 1177, 1970.
- 6 Schofield J G. Role of cyclic 3',5' AMP in release of growth hormone in vitro. *Nature* 215: 1387, 1967.
- 7 Weldon V V, Clarke W L & Pachara A S. Growth hormone release using arginine and L-d-tyrosine combination. *J Pediatr* 97: 440, 1975.
- 8 Zor U, Kaneko T, Schneider H P G, McCann S M, Lowe I P, Bloom G, Borland B & Field J B. Stimulation of anterior pituitary adenyl cyclase activity and adenosine 3',5' cyclic phosphate by hypothalamic extract and prostaglandin E1. *Proc Natl Acad Sci* 63: 918, 1969.

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Table 2 Median and range of serum T_4 , T_3 and TSH concentrations in premature infants with idiopathic respiratory distress syndrome (IRDS) and in healthy controls
 n.o. = number of samples NS = not significant

	Postnatal age in days				
	<3	3-5	6-10	11-20	21-30
Serum T_4 (nmol/l)					
IRDS					
Median	83	64	96	107	109
Range	(46-167)	(6-107)	(45-147)	(60-154)	(87-167)
No	18	9	10	20	14
Controls					
Median	147	175	155	170	131
Range	(84-89)	(152-175)	(85-170)	(80-173)	(77-234)
No	15	17	9	49	25
	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.0$	NS
Serum T_3 (nmol/l)					
IRDS					
Median	0.53	0.9	1.34	1.47	1.75
Range	(0.20-1.4)	(0.0-0.39)	(0.61-1.86)	(0.96-4.1)	(1.0-6.0)
No	14	4	7	13	10
Controls					
Median	1.0	1.31	1.9	1.78	1.83
Range	(0.51-1.33)	(0.8-1.1)	(0.37-4.03)	(0.86-4.74)	(0.66-3.77)
No	6	10	1	77	2
	$p < 0.05$	$p < 0.01$	NS	NS	NS
Serum TSH (mU/l)					
IRDS					
Median	3.9	1.5	1.7	1.1	0.7
Range	(1.0-11.8)	(1.0-6.9)	(1.0-7.5)	(1.0-9.0)	(1.0-14.6)
No	15	8	7	16	9
Controls					
Median	9.7	3.6	9.0	1.4	1.5
Range	(1.0-77.5)	(1.0-13.1)	(1.0-8.7)	(1.0-4.8)	(0.7-3.4)
No	5	10	17	37	71
	$p < 0.01$	NS	NS	NS	NS

per liter). Using routine laboratory methods the absolute concentrations of TBG, TBPA and Alb in the reference serum were 9.3 mg/l, 97 mg/l and 43,000 mg/l respectively.

In the statistical calculations the Mann-Whitney test was used (7).

RESULTS

Median body weights of the IRDS and healthy infants were comparable during the four weeks follow-up (Table 1).

Median and range (lowest respective highest value) of serum T_4 , serum T_3 and serum TSH concentrations are shown in Table 2.

In infants with IRDS serum levels of T_4 de-

creased significantly ($p < 0.05$) during the initial phase of the disease to minimal levels by day 3-5 whereupon a gradual increase in serum T_4 concentrations was observed (Table 2 and Fig. 1). In healthy premature babies serum T_4 concentration increased during the early postnatal days and reached peak values by day 3-5. Serum T_4 level was lower in the sick infants compared to controls although not statistically significant for infants aged 21-30 days.

Serum T_3 concentrations also decreased significantly ($p < 0.02$) in infants with IRDS to low values by day 3-5 (Table 2 and Fig. 1) but was followed by a rapid increase to levels

Table 1 Median and range of body weights (gram) in relation to postnatal age in premature infants with idiopathic respiratory distress syndrome (IRDS) and in healthy controls

No = number of samples NS = not significant

	Postnatal age in days				
	<1	1-5	6-10	11-20	21-30
IRDS (no = 27)					
Median	1 915	1 950	2 000	2 240	2 340
Range	(1 055-2 700)	(1 210-2 650)	(1 155-2 300)	(1 170-2 850)	(1 740-2 690)
Controls (no = 17)					
Median	2 085	2 095	2 050	2 270	2 340
Range	(850-2 800)	(850-2 615)	(1 400-2 850)	(1 170-2 960)	(1 760-3 110)
	NS	NS	NS	NS	NS

Supplementary oxygen up to an inspired oxygen concentration of 50 to 80% was adequate in 4 patients; continuous positive airway pressure (CPAP) without artificial ventilation was required in 8 infants; and respirator treatment was necessary in the remaining 15 infants. Two IRDS infants with birth weights of 1 100 g and 1 560 g and gestational age of 30 weeks died by day 4 and 10 respectively. The gestational age of the surviving IRDS infants ranged from 27 to 36 weeks and birth weights ranged from 1 055-2 700 g. Gestational age was assessed as reported previously (11).

As controls 52 healthy prematures without perinatal asphyxia or respiratory problems were studied. Gestational age ranged from 26-36 weeks and did not differ significantly from the gestational age of IRDS infants. Birth weights ranged from 840-2 800 g and the infants were matched with the IRDS patients according to comparable body weights in all age groups (Table 1). Infants of mothers who received medication that could influence thyroid hormone or thyroid hormone binding proteins were not included.

All IRDS infants received glucose and from the third day of life also electrolytes and aminoacids through umbilical catheters. The control infants were fed with human milk. Comparable amounts of fluids were given to the IRDS and healthy prematures.

Some of the infants in both groups, particularly infants with IRDS or low birth weights, received one or more transfusions (10-15 ml/kg body weight) with packed erythrocytes during the study period. In all infants blood was collected before the infusion. The next sample was obtained at least 4-5 days after any transfusion. At the time when pretransfusion level of thyroid hormones probably was reached (21).

Blood was drawn from a peripheral vein. One or two blood samples were obtained within the first week of life and the subsequent blood samples were collected at weekly intervals. The blood sampling was not complete because of the unfavorable conditions of the babies and the amount of serum necessary for determination of all thyroid variables (see tables). Parents were informed and consent obtained.

Serum concentrations of thyroxine (T_4) were determined using competitive binding microtechnique and

serum triiodothyronine (T_3) and thyrotropin (TSH) were measured by radioimmunoassay (11-17). The thyroxine binding globulin (TBG), prealbumin (TBPA) and albumin (Alb) were determined using rocket immunoelectrophoresis as previously reported (14). The thyroxine binding protein concentrations were presented in terms of those obtained in a reference serum (100 arbitrary units).

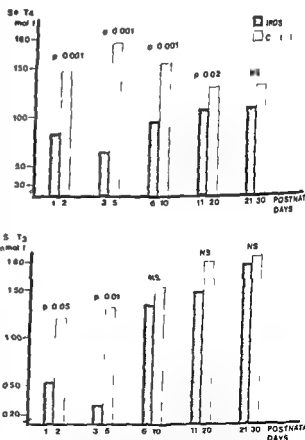


Fig 1 Changes in serum concentrations of thyroxine (T_4) and triiodothyronine (T_3) in premature infants with idiopathic respiratory distress syndrome (IRDS) and healthy controls. The bars indicate median values for each age group of infants. NS = not significant.

Table 3 Median and range of serum TBG, TBPA and Alb concentrations in premature infants with idiopathic respiratory distress syndrome and in controls
No = number of samples. NS = not significant

	Postnatal age in days				
	<3	3-5	6-10	11-20	21-30
Serum TBG (arbitrary units)					
IRDS					
Median	118	116	133	161	134
Range	(90-179)	(86-176)	(99-107)	(96-198)	(105-176)
No	16	7	7	15	9
Controls					
Median	157	166	179	167	174
Range	(74-234)	(118-241)	(141-229)	(96-210)	(187-231)
No	21	10	11	19	15
	$p < 0.01$	$p < 0.01$	NS	NS	$p < 0.05$
Serum TBPA (arbitrary units)					
IRDS					
Median	5	10	37	44	38
Range	(15-47)	(13-36)	(11-59)	(75-39)	(34-56)
No	16	5	6	13	8
Controls					
Median	6	6	24	30	33
Range	(10-36)	(16-37)	(19-40)	(18-47)	(11-53)
No	20	10	9	16	13
	NS	NS	NS	$p < 0.001$	NS
Serum Alb (arbitrary units)					
IRDS					
Median	67	78	73	75	75
Range	(51-80)	(63-97)	(66-86)	(65-81)	(71-83)
No	16	7	6	14	9
Controls					
Median	76	73	87	75	83
Range	(57-113)	(49-101)	(66-106)	(55-96)	(67-101)
No	21	10	11	18	14
	NS	NS	NS	NS	NS

were not significantly different in the two groups of infants (Table 3)

TBG possesses binding sites of much higher affinity for T_4 and T_3 than those of TBPA and Alb (22) and the increased serum TBG level determines the serum thyroid hormone concentrations in newborns (14). In attempting to evaluate the saturation of TBG binding sites the individual ratios between serum concentrations of thyroid hormones and TBG were calculated. Table 3 shows that median values of T_4 /TBG ratios were significantly lower in infants with IRDS than in healthy infants aged

3-20 days. The T_3 /TBG ratios were not significantly different in the two groups although the same tendency could be observed.

DISCUSSION

The present study demonstrates pronounced changes in serum concentrations of thyroid hormones and hormone binding proteins during the acute and recovery stages of IRDS.

The findings of low serum T_4 and serum T_3 concentrations in IRDS infants during the first 2-3 days of life confirm previous observa-

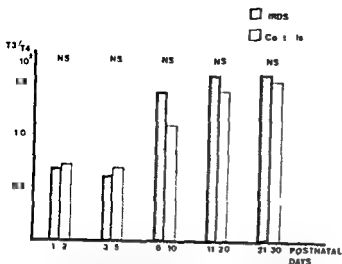


Fig 2 The ratio between serum concentrations of triiodothyronine (T_3) and thyroxine (T_4) in each age group of infants. Median values are shown. Symbols as in Fig 1.

which did not differ significantly from those of healthy prematures (by day 6-10). In healthy babies a continuous rise in serum T_3 occurred during the study period. The ratio between serum T_3 and serum T_4 concentrations were not significantly different in the two groups of in-

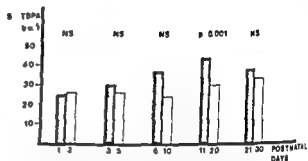
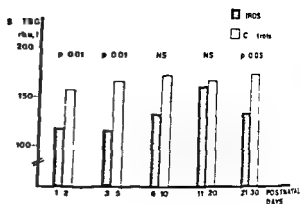
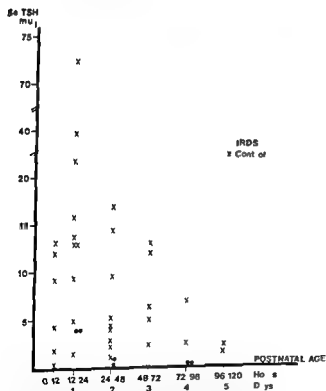


Fig 4 Changes in serum concentrations of thyroxine binding globulin (TBG) and prealbumin (TBPA) in each age group of healthy and sick premature infants. Median values are shown. Symbols as in Fig 1.



T_3 conversion in IRDS infants (1-5-17) were not confirmed by the present calculations of serum T_3/T_4 ratios in healthy and sick infants (Fig. 2) but a tendency towards lower T_3/T_4 values in IRDS compared to healthy infants was observed during the first 5 days of life. Serum reverse T_3 was not measured but Klein et al. (17) have recently reported data indicating a decrease in serum T_3 /reverse T_3 ratios in IRDS infants between 12 and 72 hours of age.

Following recovery from IRDS serum T_3 concentrations increased and reached T_3 levels of healthy prematures by day 6-10 whereas the serum T_4 increase was more gradual possibly indicating an increased T_4 to T_3 conversion during the recovery stage of IRDS. The T_3/T_4 ratios were similar to full terms and adults (12-18). In earlier reports on thyroid function in patients recovering from IRDS (1-17) the pattern of the changes in serum concentrations of T_3 and T_4 were comparable to those observed in the present study. A comparison between the absolute serum concentrations would not be reasonable due to the very heterogeneous population with respect to body weights, a factor known to influence the T_4 concentration in blood (7).

The serum concentrations of thyroid hormone binding proteins also changed markedly in infants during recovery from IRDS where as only minor changes appeared in the healthy infants during the study period (Fig. 4). In a report by Hardie et al. (10) serum concentrations of total protein, albumin and IgG globulin were found to be lower in IRDS patients than in healthy prematures.

The mechanisms that determine the individual changes in serum concentrations of the thyroid hormone binding proteins are unknown but differences with respect to synthesis or utilization of the proteins are suggested. The low serum TBG concentrations in IRDS infants within the first days of life might indicate an association between the perinatal production of pulmonary surfactant and the TBG synthesis. This does not necessarily indicate a

causal relationship and further investigations should be made. The rapid and pronounced rise in serum TBPA in IRDS infants might possibly be caused by the increased adrenal response and higher plasma corticoid levels which have been observed in IRDS patients compared with healthy neonates (2-3) since glucocorticoids induce an increase in serum TBPA without changing serum TBG concentrations significantly (4) or reduce serum TBG binding capacity (20).

The present study provides evidence for the pronounced changes in thyroid hormones and hormone binding protein concentrations during acute and recovery stages of IRDS but the pathogenetic significance of these alterations is not clear. Studies in fetal and adult animals have demonstrated the influence of thyroid hormones upon lung growth and surfactant production (8-23-29). Intraamniotic thyroid hormone application in high risk pregnancies (25) or temporary T_4 or T_3 administration to IRDS patients have been proposed (6). A supportive thyroid hormone therapy is in accordance with our suggestions of a reduced saturation of the serum thyroid hormone binding proteins. Clinical trials are needed to determine whether such an early depression of thyroid function has adverse consequences. Administration of thyrotropin releasing hormone (11) should also be taken into consideration.

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REFERENCES

1. Abbassi V, Merchant K & Abramson JJ. Postnatal triiodothyronine concentrations in healthy pre-

Table 4 Median and range of the ratios between serum concentrations of thyroid hormone and thyroxine binding globulin (TBG) in infants with idiopathic respiratory distress syndrome (IRDS, and in healthy controls

No = number of samples NS = not significant

	Postnatal age in days				
	<3	3-5	6-10	11-20	21-30
<i>T₄/TBG</i> × 10 ⁴					
IRDS					
Median	50	38	67	98	145
Range	(13-152)	(31-45)	(51-103)	(54-141)	(61-179)
No	13	2	6	11	9
Controls					
Median	62	70	78	115	116
Range	(16-112)	(40-82)	(61-150)	(77-150)	(86-167)
No	19	5	7	16	14
	NS	NS	NS	NS	NS
<i>T₃/TBG</i> × 10 ³					
IRDS					
Median	79	57	50	65	76
Range	(47-147)	(16-68)	(43-76)	(34-141)	(47-107)
No	16	7	7	15	9
Controls					
Median	86	95	101	87	75
Range	(50-151)	(58-137)	(45-131)	(46-126)	(53-127)
No	21	10	11	19	15
	NS	<i>p</i> < 0.001	<i>p</i> < 0.001	<i>p</i> < 0.01	NS

tions (1, 5, 6, 17, 24). In addition the study provides evidence that a decrease in thyroid hormone concentrations occurs during the acute stage of IRDS. This is in contrast to the early postnatal rise in thyroid hormone serum levels which appears in healthy babies due to the TSH surge (9, 12). In IRDS infants the TSH stimulation of the thyroid gland is probably less pronounced since the serum TSH levels during the first days after birth were significantly lower than in healthy pretermatures. Similar TSH findings have recently been reported by Cuestas & Engel (6) and Klein *et al* (17). Later in infancy serum TSH concentrations were similar in the two groups of infants and therefore cannot explain the ensuing changes in serum T_4 and T_3 concentrations.

The serum TBG concentrations were significantly lower in infants during the acute stage of IRDS than in healthy pretermatures whereas

serum TBPA and Alb levels were comparable in the two groups of infants confirming our preliminary results (13, 15). The low serum TBG level contribute to explanation of the diminished serum thyroid hormone concentrations in IRDS patients. Calculations of the ratio between serum concentrations of thyroid hormones and TBG also indicate more unsaturated binding sites upon TBG in IRDS infants than in healthy pretermatures (Table 4). Redding & Pereira (24) and Cuestas & Engel (6) reached the same conclusion by evaluating the free thyroxine index. It is not known whether a reduced thyroid hormone secretion or an increased utilization of T_4 and T_3 occur during the initial phase of IRDS. Findings of low oxygen consumption in IRDS infants compared to healthy pretermatures (19) agree with the hypothesis of a decrease in the activity of the thyroid gland.

Previous suggestions of a decreased T_4 to

THE EFFECT OF FEEDS OF DIFFERING COMPOSITION ON ENTERO INSULAR HORMONE SECRETION IN THE FIRST HOURS OF LIFE IN HUMAN NEONATES

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KEY WORDS Human milk dextrose glucose insulin glucagon enteroglucagon gastrin gastric inhibitory peptide newborn

In recent years the gut has emerged as an important endocrine organ producing a series of gastro intestinal hormones (18) The development of radioimmunoassay techniques for the measurement of these hormones in plasma has permitted *in vivo* physiological studies (3) In the neonate the physiological release of gut hormones in response to feeding may play an important role in the adaptation to extra uterine nutrition (*vide infra*) However although some gastro intestinal hormones have been shown to be present in the foetal gut (13 17) there have been few studies demonstrating the physiological stimuli for gut hormone release in the newborn infant

As part of a previous study we examined the effect of the first feed of breast milk on the release of gastrin enteroglucagon and gastric inhibitory polypeptide (GIP) together with the effects on plasma insulin and on blood glucose (2) In the present study we compare these ef-

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METHODS

Twenty-one infants at or near to term were studied with the approval of the ethics committee All had been admitted to the Special Care Baby Unit and all had indwelling umbilical arterial catheters for clinical monitoring purposes They were nursed in incubators at an environmental temperature appropriate for their weight and gestation None had a rectal temperature of $<36^{\circ}\text{C}$ none had a pH <7.35 P_{aO_2} was maintained in the range 8-12 kPa (60-90 mmHg) and the clinical condition of the infants was stable throughout the study Infants were randomly allocated into two groups

Group I comprised 9 infants who received a first feed of 10% dextrose (*vide infra*) Their mean birth weight was 2570 g (range 1070-3090 g) and mean gestational age 36.9 weeks (range 36-40 weeks) Five had mild to moderate respiratory distress 3 of whom required additional inspired oxygen The other 4 had moderate birth asphyxia requiring transient endotracheal intubation oxygen and alkali therapy

Group II comprised 12 infants who received a first feed of human milk (*vide infra*) Their mean birthweight was

- term infants and in infants with respiratory distress syndrome *Pediatr Res* 11 802 1977
- 2 Bacon G F George R Koell B T & Howatt W F Plasma corticoids in the respiratory distress syndrome in normal infants *Pediatrics* 55 500 1975
 - 3 Ballard R A & Ballard P I Use of prenatal glucocorticoid therapy to prevent respiratory distress syndrome *Am J Dis Child* 130 982 1976
 - 4 Burr W A Ramsden D B Griffiths R S Black F G & Hoffenberg R Meinhold H & Wenzel K W Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones *Lancet* II 58 1976
 - 5 Cuestis H A Lindall A & Engel R R Low thyroid hormones and respiratory distress syndrome of the newborn. Studies on cord blood *Am J Med* 295 297 1976
 - 6 Cuestis R A & Engel R R Low thyroid activity in prematures with RDS (Abstract) *Pediatr Res* 11 474 1977
 - 7 Dussault J H Monette J Fiset P Laberge E & Laberge C Factors influencing results for thyroxine concentration in blood as measured in paper filter spots in a screening program for neonatal hypothyroidism *Clin Chim* 27 1392 1976
 - 8 Erenberg A Omori K Menkes J H Oh W & Fisher D A Growth and development of the thyroidectomized ovine fetus *Pediatr Res* 8 783 1974
 - 9 Erenberg A Phelps D L Lam M & Fisher D A Total and free thyroid hormone concentrations in the neonatal period *Pediatrics* 53 211 1974
 - 10 Hardie G Harrison V C & Kench J E Further observations on serum proteins in respiratory distress syndrome of the newborn *Arch Dis Child* 43 471 1968
 - 11 Jacobsen B B Andersen H J Dige Petersen H & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand* 66 541 1977
 - 12 Jacobsen B B Andersen H J Petersen B Dige Petersen H & Hummer L Serum levels of thyrotropin, thyroxine and triiodothyronine in fullterm small for gestational age and preterm newborn babies *Acta Paediatr Scand* 66 681 1977
 - 13 Jacobsen B B Petersen B & Andersen H J Serum levels of thyroxine binding proteins in infants with respiratory distress syndrome. The 16th annual meeting of the European Society for Pediatric Endocrinology. Session for recent results. Cambridge 1977
 - 14 Jacobsen B B Petersen B Andersen H J & Hummer L Serum concentrations of thyroxine binding globulin, prealbumin and albumin in healthy fullterm small for gestational age and preterm newborn infants *Acta Paediatr Scand* 68 49 1979
 - 15 Jacobsen B B & Petersen B Comparisons between serum concentrations of thyroxine and thyroxine binding proteins in samples simultaneously obtained from capillary peripheral vein, central vein and aorta in newborn infants *Acta Paediatr Scand* 68 43 1979
 - 16 Kamper J Bækgaard P Petersen B Christensen P M Tygstrup I & Fris Hansen B Behaviour of respiratory distress syndrome med overtryk luftvejene (continuous positive airway pressure) *Ugeskr Læger* 135 2207 1973
 - 17 Klein A H Foley B Ho R S Kenny F M & Fisher D A Thyroid response to parturition. Relationship to gestational age and the respiratory distress syndrome (Abstract) *Pediatr Res* 11 477 1977
 - 18 Klein A H Stinson D Foley H Larsen P R & Foley T P Thyroid function studies in preterm infants recovering from the respiratory distress syndrome *J Pediatr* 91 261 1977
 - 19 Levison H Delipapadoulou M & Sawyer P R Oxygen consumption in newly born infants with the respiratory distress syndrome *Biol Neonate* 7 196 1964
 - 20 Oppenheimer J H & Werner S Effect of prematurity on thyroxine binding proteins *J Clin Endocrinol* 26 715 1966
 - 21 Paletta B Rossipal E & Haidvogel M Veränderung des PBI Spiegels bei Neugeborenen nach Austauschtransfusion *Pediatr Paedol* 5 339 1969
 - 22 Prince H P & Ramsden D B A new theoretical description of the binding of thyroid hormones to serum proteins *Clin Endocrinol* 7 307 1977
 - 23 Redding R A & Douglas W H J Thyroid hormone influence upon lung surfactant metabolism *Science* 175 994 1972
 - 24 Redding R A & Pereira C Thyroid function in respiratory distress syndrome of the newborn *Pediatrics* 54 423 1974
 - 25 Sick J Mitsuuchi S Barku G Lunenfeld B Brish M Goldman H & Serr D M Intramammary thyroxine effect in high risk pregnancies. The 16th annual meeting of the European Society for Pediatric Endocrinology. Cambridge 1977
 - 26 Schaffer A J & Avery M E Diseases of the newborn. Saunders Philadelphia London and Toronto 1977 4th ed p 134
 - 27 Siegel S Nonparametric statistics for the behavioral sciences McGraw Hill Kogakusha Tokyo 1966
 - 28 Smith D W Klein A M Henderson J R & Myrianthopoulos N C Congenital hypothyroidism: signs and symptoms in the newborn period *J Pediatr* 87 958 1975
 - 29 Wu B Kikkawa Y Orzalesi M M Motoyama E K Kaibara M Zigs C J & Cook C D The effect of thyroxine on the maturation of fetal rabbit lungs *Biol Neonate* 22 161 1973

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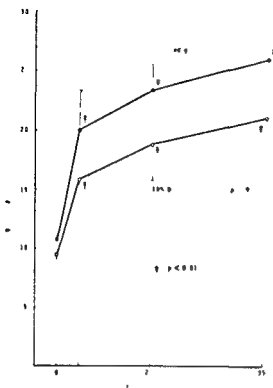


Fig. 1 The effect of the first feed of 10% dextrose or breast milk on plasma gastrin (\pm S.E.M.)

2945 (range 2110–3940) and a mean gestational age of 37.8 weeks (range 37–39 weeks). Nine infants were suffering from moderately severe respiratory distress requiring enriched inspired oxygen. Of the other 3, one was a breech delivery with severe birth asphyxia and 2 had lesser degrees of birth asphyxia requiring transient endotracheal intubation, oxygen and alkali therapy.

Feeding procedure

The first feed was given between 4 and 6 hours of age via a nasogastric tube which was passed at least 2 hours before feeding. Group I received a 5 ml/kg bolus of 10% dextrose. Group II a 5 ml/kg bolus of breast milk. This volume gives 60 ml/kg per 24 hours with a 2 hourly feeding regime. The feed was given by gravity over 2–4 min with the infant lying on the right side with the head of the mattress raised. Infants were not disturbed during the study period. At the end of the test the stomach contents were re-aspirated and in no case was more than 25% of

the original feed volume returned. Regurgitation of the feed did not occur in any instance.

Blood sampling and assay methods

Blood samples were drawn from an indwelling umbilical artery catheter immediately before the feed and at 5, 25 and 55 min after the feed. Samples were analysed for plasma gastrin, pancreatic glucagon, enteroglucagon, enteroinhibitory polypeptide (GIP) and insulin and for blood glucose. The details of the sampling procedure and of the assay methods used are described in our previous publication (2). Results were assessed for significance by means of Student's *t* test assuming equal variance.

RESULTS

Gut hormones

In the 10% dextrose fed group the mean basal plasma gastrin level was 9.4 ± 2.2 (S.E.M.) pmol/l, rising immediately and progressively to 21.3 ± 7.7 pmol/litre at 55 min following the feed ($p < 0.01$) and in the milk fed group gastrin rose from a fasting level of 10.9 ± 1.1 pmol/l to 26.1 ± 1.9 pmol/l at 55 minutes ($p < 0.01$). There were no significant differences between the mean gastrin level in the two groups either before or at 5, 25 or 55 min after the feed (Fig. 1).

The mean basal plasma GIP level was 126 ± 27 (S.E.M.) pg/ml in the 10% dextrose fed group and 118 ± 32 pg/ml in the milk fed group. In neither group was there a significant change in level after feeding (Table 1).

Mean basal enteroglucagon level was 139 ± 16 (S.E.M.) pg/ml equivalent in the 10% dextrose fed group and there was no significant change with feeding. In contrast in the milk fed group there was a significant rise in enteroglucagon level from 145 ± 30 pg/ml equivalent to 306 ± 65 at 55 min ($p < 0.05$) which was

Fig. 1 Effect of the first feed of 10% dextrose compared with breast milk on plasma gastric inhibitory polypeptide (pg/ml \pm S.E.M.)

Time (min)	0	5	25	55
10% dextrose fed group <i>n</i> = 5	126 ± 27	165 ± 18 N.S.	186 ± 43 N.S.	170 ± 25 N.S.
Milk fed group <i>n</i> = 8	118 ± 32	203 ± 79 N.S.	169 ± 38 N.S.	154 ± 28 N.S.

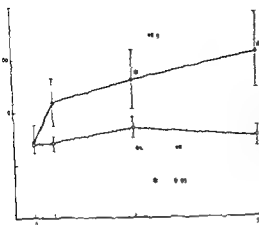


Fig. 2 The effect of the first feed of 10% dextrose or breast milk on plasma enteroglucagon (\pm S.E.M.)

significantly higher than the level attained at 55 min in the 10% dextrose fed group ($p < 0.05$) (Fig. 2). Mean basal plasma pancreatic glucagon was 57 ± 12 pmol/l in the 10% dextrose group and 65 ± 12 pmol/l in the milk fed group. There was no change in level in either group following the feed and there were no differences in levels between the two groups (Table 2).

Plasma insulin and blood glucose

The mean basal plasma insulin level in the 10% dextrose fed group was 5.1 ± 1.2 (S.E.M.) mU/l and a significant rise occurred following the feed reaching 11.0 ± 1.7 mU/l at 55 min ($p < 0.01$). In the milk fed group basal plasma insulin was 6.8 ± 1.3 mU/l rising to 15.3 ± 3.2 at 55 min ($p < 0.01$). There were no significant differences between the two groups in mean

insulin levels before and at 5, 25 and 55 min after the feed (Fig. 3).

Mean basal blood glucose concentration in the 10% dextrose fed group was 3.73 ± 0.22 mmol/l rising to 4.93 ± 0.56 at 55 min ($p < 0.01$). In the milk fed group mean basal blood glucose was 3.67 ± 0.22 mmol/l rising to 4.51 ± 0.25 at 55 min ($p < 0.01$). The glycaemic responses in the two groups did not differ significantly (Fig. 4).

DISCUSSION

After birth enteral feeding triggers several important structural and physiological developmental changes in the neonate which may be considered to be a part of his adaptation to extra uterine nutrition. In piglets (20, 23) and rats (14) feeding results in marked structural changes and growth of the digestive tract and in piglets (23) jejunal lactase and acid phosphatase activities are enhanced. In addition it has been demonstrated that early feeding increases the responsiveness to glucose of pancreatic β cells in neonatal rats (1) and piglets (7). It is suggested that the intermediary link between feeding and the changes described above may be in part the release of gut hormones. In support of this hypothesis is the trophic action of some gut hormones in adult animals and man: gastrin (10, 11) and enteroglucagon (8) stimulate structural development of the gut and cholecystokinin (15) and gastrin (16) stimulate the growth of the exocrine pancreas. Moreover, Lichtenberger (14) has shown in neonatal rats that feeding produces rises in plasma gastrin which parallel the induced gastrointestinal changes. Plasma

Table 2 Effect of the first feed of 10% dextrose compared with breast milk on plasma pancreatic glucagon (pmol/l) (\pm S.E.M.)

Time (min)	0	5	25	55
10% dextrose fed group <i>n</i> = 9	57 ± 17	68 ± 15	63 ± 12	63 ± 11
Milk fed group <i>n</i> = 11	65 ± 17	68 ± 12	73 ± 13	67 ± 13

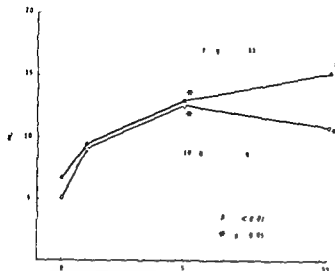


Fig. 3 The effect of the first feed of 10% dextrose or breast milk on plasma insulin (\pm S.E.M.)

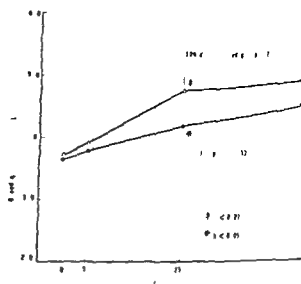


Fig. 4 The effect of the first feed of 10% dextrose or breast milk on blood glucose (\pm S.E.M.)

gastrin together with enteroglucagon are known to increase during the first 4 days of life in the human neonate (19). Von Berger (22) has shown that gastrin increases following the first milk feeding; however, the effect of feeding on other gut hormones in the neonate has received little attention. In the present study we have examined the effect of two enteral stimuli, 10% dextrose and human milk, given to previously unfed sick neonates on the release of three gut hormones (gastrin, enteroglucagon and GIP) together with the effects on plasma insulin and on blood glucose.

The gastrin responses to the first feed of human milk and of 10% dextrose were similar with a significant rise at 5, 25 and 55 min following the feed. In adults, digested protein is a potent stimulus for gastrin release; it is therefore interesting that milk feeding did not produce a significantly greater response than 10% dextrose feeding. This could be explained by the absence of sufficient proteolysis during the 55 min study period following the feed or might be due to a failure of neonatal gastrin-secreting cells to respond to the products of protein digestion. Gastric distension may well have been the main stimulus to gastrin release in this study.

Enteroglucagon levels were significantly raised at 25 and 55 min following the first feed

of human milk, though the fasting level was unchanged by a 10% dextrose feed. In adults, enteroglucagon release is strongly stimulated by carbohydrate and moderately stimulated by long chain triglycerides (9). It was therefore surprising that there was no enteroglucagon response to a 10% dextrose feed, but this may be because glucose is rapidly absorbed in the upper small intestine, whereas enteroglucagon is mostly found in the ileum. It is also possible that in the neonate, triglyceride is a more potent stimulus to enteroglucagon release than in the adult, perhaps as an adaptation to the high fat intake of the milk-fed infant.

Gastric inhibitory polypeptide did not increase significantly after a milk or 10% dextrose feed, though in both cases postprandial levels tended to be higher than fasting levels. In adults, the GIP response to feeding is biphasic, with an early peak at 45 min in response to carbohydrate, followed by a late plateau at 2–3 hours in response to fat (4). Since our postprandial study period lasted only 55 min, we might not have seen a delayed fat response. We failed to achieve a significant early GIP rise even with 10% dextrose, which has a higher carbohydrate content than milk. GIP is known to release insulin in adults (5) and King et al. (12) have speculated that their failure to enhance the insulin response to enteral

compared with parenteral glucose in neonates was due to a failure of responsiveness of neonatal pancreatic B cells to GIP. In view of our findings we would like to propose an alternative hypothesis that the failure of gastrointestinal enhancement of the insulin response to glucose is due to a failure of early feeding to release GIP and it is possible that the increasing glucose response to insulin induced by later feeds (1-7) is due to the initiation of GIP release.

The glycaemic response to 10% dextrose and milk feeding was not significantly different in spite of the high carbohydrate content of the former. The insulin response to 10% dextrose appeared less than that to a milk feed but this difference was not significant. Gastrin (6), enteroglucagon (21) and GIP (5) may all be stimuli to insulin release in adults. In our study gastrin and GIP responses were similar in milk fed and 10% glucose fed neonates whereas enteroglucagon was stimulated only by milk feeding. If enteroglucagon were a potent stimulus of insulin release in the neonate a greater insulinaemic response would be expected in the milk fed group. There was in fact a higher insulin:glucose ratio in the milk fed group 55 min after feeding (mean ratio 3.8) than in the 10% dextrose fed group (mean ratio 2.1). However since this difference was not significant we have failed to demonstrate that enteroglucagon is an important effector in the enteroinsular axis in newborn infants.

It is concluded that gut hormone release shortly after birth may differ from that in adults not only in the quantity of response evoked by an enteral stimulus but in the quality of stimulus required to produce a response. However it is possible that these differences may relate to the fact that our studies were confined to sick neonates.

Further work is needed to define the gut hormone responsiveness of well and ill preterm and full term neonates at different postnatal ages fed in different ways since this information may provide a rational basis for designing optimal feeding regimes which will

stimulate most effectively high risk neonates in their physiological adaptation to feeding.

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REFERENCES

- Asplund K. Effects of postnatal feeding on the functional maturation of pancreatic islet B cells of neonatal rats. *Diabetologia* 8: 153, 1972.
- Aynsley Green A, Bloom S R, Williamson D H & Turner H C. Endocrine and metabolic response in the human newborn to first feed of breast milk. *Arch Dis Child* 52: 4, 191, 1977.
- Bloom S R. Radioimmunoassay of intestinal hormones. *Gut* 15: 407, 1974.
- Brown J C, Dryburgh J R, Frost J L, Otte S C & Pederson R A. Properties and actions of GIP. In S R Bloom (ed.) *Gut Hormones*. Churchill Livingstone, Edinburgh, 1978, pp. 177-281.
- Dupre J, Ross S A, Watson M & Brown J C. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 37: 876, 1973.
- Dupre J, Curtis J D, Unger R H, Waddell R W & Beck J C. Effects of secretin, pancreozymin or gastrin on the response of the endocrine pancreas to administration of glucose of arginine in man. *J Clin Invest* 48: 743, 1969.
- Gentz J, Persson B, Kellum M, Bengtsson G & Thorell J. Effect of feeding on intravenous glucose tolerance and insulin response in piglets during the first day of life. *Life Science* 10: 1237, 1971.
- Gleeson M H, Bloom S R, Polak J M, Henry K & Dowling R M. An endocrine tumour in kidney affecting small bowel structure, motility and absorptive function. *Gut* 12: 733, 1971.
- Holst J J, Christiansen J & Kuhl C. The enteroglucagon response to intrajejunal infusion of glucose, triglycerides and sodium chloride. *Scand J Gastroenterol* 11: 797, 1976.
- Johnson L R. The trophic action of gastrointestinal hormones. *Gastroenterology* 70: 278, 1976.
- Johnson L R. New aspects of the trophic action of gastrointestinal hormones. *Gastroenterology* 77: 88, 1977.
- King K C, Schwartz M, Yamaguchi K & Adam P A J. Lack of gastrointestinal enhancement of the insulin response to glucose in newborn infants. *J Pediatrics* 91: 5783, 1977.
- Larsson L I, Rehfeld J I & Gøtztermann M. Gastrin in the human fetus. *Scand J Gastroenterol* 12: 869, 1977.
- Lichtenberger L & Johnson L R. Gastrin in the

- autogenic development of the small intestine *Am J Physiol* 227 2 390 1974
- 15 Munz D L, Black D & Webster P D Hormonal control of pancreatic growth *J Clin Invest* 53 2300 1973
 - 16 Mayston P D & Burrowman J A The influence of chronic administration of pentagastrin on the rat pancreas *Am J Exp Physiol* 56 113 1971
 - 17 Moxey P C & Trier J S Development of endocrine cells in the human fetal small intestine *Gastroenterology* 72 4 p 819 (abstracted) 1977
 - 18 Pearse A G I, Polak J M & Bloom S R The newer gut hormones *Gastroenterology* 72 746 1977
 - 19 Rogers I M, Davidson D C, Lawrence J, Ardill J & Buchanan K D Neonatal secretion of gastrin and glucagon *Arch Dis Child* 49 796 1974
 - 20 Stoddart H W & Widdowson E M Changes in the organs of pigs in response to feeding for the first 24 hours after birth III Fluorescence Histochemistry of the Carbohydrates of the Intestine
 - 21 Unger R H, Ohneda A, Valverde I, Eisentrut A M & Exton J Characterisation of the responses of circulating glucagon like immunoreactivity to intra duodenal and intravenous administration of glucose *J Clin Invest* 47 48 1968
 - 22 Von Berger L, Hennrich I, Raptis S, Heinze E, Jonathan W, Teller W M & Pfeiffer E F Gastrin concentration in plasma of the neonate at birth and after the first feeding *Paediatrics* 68 764 1976
 - 23 Widdowson E M, Colombo V E & Artavanis C A Changes in the organs of pigs in response to feeding for the first 24 hours after birth II The digestive tract *Biol Neonate* 28 272 1976

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SHORT COMMUNICATION

FAECAL LEUCOCYTES IN CAMPYLOBACTER ASSOCIATED
DIARRHOEA IN INFANTS

Campylobacters have recently been recognized as important causative agents of human diarrhoea in all age groups (1). In the study of Skirrow (5) campylobacters were found to account for 7.1% of all unselected cases of diarrhoea. The clinical picture varies but abdominal pains and the presence of blood pus and mucus in the stools have been reported. These features are typical of exudative diarrhoea where polymorphonuclear leucocytes (PMNL) are often found in the faeces.

We have attempted to isolate campylobacters from all patients admitted for diarrhoea to the Department of Paediatrics at the Tampere Central Hospital over a period of 5 months beginning in May 1978. At the same time we have looked for PMNL in the stools. The preparation for PMNL was made by smearing mucus from a fresh stool specimen onto a glass slide which was dried in the air, stained and examined microscopically (Fig. 1). The culture method for campylobacters was adapted from Skirrow (5) and Lauwers et al. (3).

Campylobacters were isolated in 5 cases during the 5 month period among 141 patients hospitalized for diarrhoea (3.5%). Faecal

leucocytes were demonstrated in 20 cases (14.2%). Of the 5 campylobacter positive patients PMNL were found in the faeces of 4 cases. Therefore campylobacters accounted for 20% of all cases of exudative diarrhoea in this series. In addition we saw a case of campylobacteriosis in a 9-day old infant with PMNL in the faeces before the systematic screening for campylobacters was begun. We have included this case in Table 1.

The main clinical characteristics of campylobacter associated disease in the 6 infants are presented in Table 1. The diarrhoea was exudative in 5 cases with either gross blood or mucus or both present. With the exception of Case 1 (9 day old infant) all the other patients remained in good condition despite prolonged diarrhoea. Their routine laboratory tests remained unremarkable. In no case was there vomiting associated with diarrhoea but at least two of the patients experienced abdominal pains.

Faecal leucocytes are seen in exudative diarrhoea which usually is caused by invasive intestinal pathogens i.e. *Salmonella*, *Shigella*, enteroinvasive strains of *E. Coli* (2) and *Yer*

Table 1. Clinical characteristics of six cases of campylobacter associated diarrhoea in infants

Case	Age	Duration of symptoms (days)	Fever (°C)	WBC	Quality of stools	PMNL in stools
1	9 days	3	39.6	15 400	Mucoid	+
	1 months	11	37.6	11 400	Mucoid bloody	+
3	1 months	9	38.4	7 900	Watery	-
4	14 months	10	38.3	12 800	Mucoid bloody	+
5	1 months	8	40.0	8 000	Mucoid bloody	+
6	4 months	20	37.8	4 400	Watery bloody	+

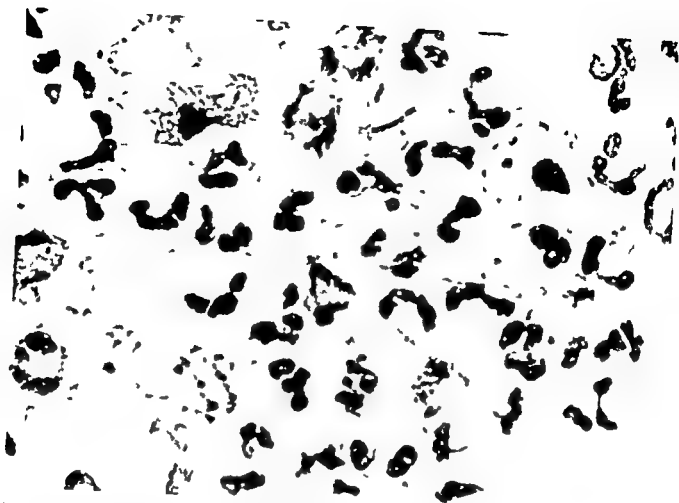


Fig. 1 Fecal leucocytes from a case of campylobacter associated diarrhoea. Mucus from the stools was smeared on a glass slide, dried and stained with Gram stain. $\times 1000$.

sinia enterocolitica (4). We also looked for these bacteria in the present patients with negative results. It now seems that campylobacters may be an important cause of exudative diarrhoea in our community. Campylobacteriosis appeared to be endemic as there was no history of travelling abroad in these patients. In any case campylobacters must be sought along with other intestinal pathogens in exudative diarrhoea of infants.

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REFERENCES

- 1 Editorial. Campylobacter enteritis. *Lancet* II 135 1978.
- 2 Hays J C, DuPont H L & Hornick R B. Fecal leukocytes in diarrheal illness. *Ann Intern Med* 76: 697 1972.
- 3 Lauwers S, DeBoek W & Butzler J P. Campylobacter enteritis in Brussels. *Lancet* I 604 1978.
- 4 Maki M, Gronroos P & Vesikari T. In vitro invasiveness of *Yersinia enterocolitica* isolated from children with diarrhea. *J Infect Dis* in press.
- 5 Skirrow M B. Campylobacter enteritis: a new disease. *Br Med J* II 9 1977.

SHORT COMMUNICATION

LYSOZYME AND COMPLEMENT FACTORS IN SERA FROM CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

It has been suggested that serial determinations of serum lysozyme may be of value in detecting early systemic relapses of acute lymphoblastic leukemia (ALL) (4-7). This supposition is based on the finding that the concentration of serum lysozyme in children with ALL fluctuates with the course of the disease being low before treatment and during relapse (4-5-7). Similarly high serum concentrations of the C1 esterase inactivator (C1 IA) and of the complement factor C4 are found in patients with malignant diseases including leukemia with a tendency to normalization during successful cytostatic treatment or after surgical removal of the cancer (2). Thus in malignancies the serum levels of C1 IA and of C4 apparently parallel disease activity (1-2).

In order to assess whether changes in the serum concentrations of lysozyme and of the complement factors C1 IA, C3 and C4 could warn about impending relapse in ALL, we have made serial determinations of these serum components in 30 children with ALL, age 2-14 years. All children received conventional cytostatic treatment (6). Serum lysozyme concentration was determined by a nephelometric method (3). Serum C1 IA, C3 and C4 were determined by single radial immunodiffusion.

Low serum lysozyme concentrations were found in children with untreated ALL and in children with hematological relapses of the disease as other workers have reported (4-5). The mean serum lysozyme value in these patients were 2.14 mg/l (ranges 0.6-5 mg/l). These values were significantly different ($p < 0.05$) from those found in patients in remission on treatment: mean 4.13 mg/l (ranges 1.0-10.9 mg/l) off treatment: mean 4.60 mg/l (ranges 0-10.0 mg/l) and in healthy controls: mean 4.33 mg/l (ranges 2.97-7.0 mg/l). Five patients relapsed during the observation period. Their serum lysozyme values prior to, during and following relapses are given in Fig. 1. Three of the five patients had abnormally low serum lysozyme values at the time of relapse but only one of the five patients had low values 1 month prior to the relapse. In agreement with reports from Finch *et al.* (5) we found abnormally low lysozyme concentrations in sera from patients in remission with signs of bone marrow hypoplasia secondary to cytostatic treatment.

The mean serum concentration of C1 IA in children with ALL before treatment or in relapse was 0.48 g/l (Fig. 2). In children in remission either on or off treatment the mean serum concentration of C1 IA was 0.38 g/l (Fig. 2). The corresponding

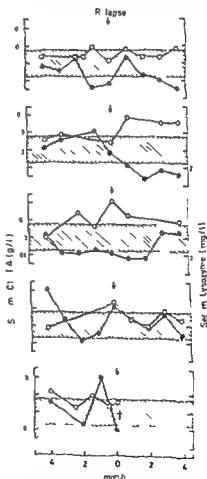


Fig. 1 The serum concentrations of lysozyme (●) and C1-esterase inactivator (○) in 5 children with acute lymphoblastic leukemia before, during and following bone marrow relapse (indicated by arrows). One of the patients died (*) shortly after the relapse had been diagnosed. Shaded area = reference ranges.

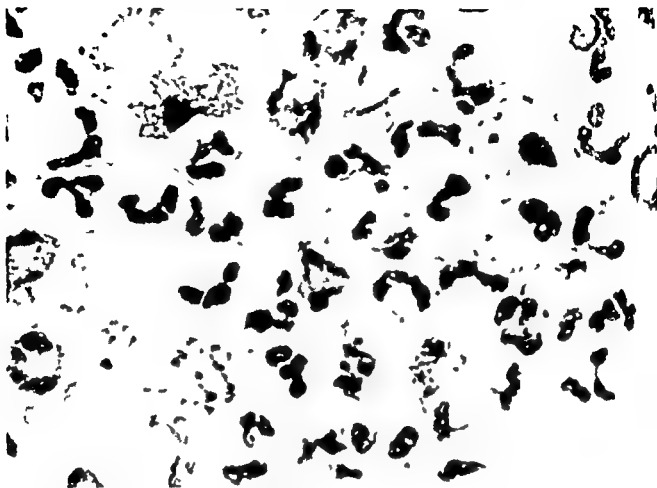


Fig. 1. Faecal leukocytes from a case of campylobacter associated diarrhoea. Mucus from the stools was smeared on a glass slide, dried and stained with Gram stain $\times 1000$.

Yersinia enterocolitica (4). We also looked for these bacteria in the present patients with negative results. It now seems that campylobacters may be an important cause of exudative diarrhoea in our community. Campylobacteriosis appeared to be endemic: there was no history of travelling abroad in these patients. In any case, campylobacters must be sought along with other intestinal pathogens in exudative diarrhoea of infants.

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REFERENCES

- 1 Editorial. Campylobacter enteritis. *Lancet* II 134 1978.
- 2 Harris J C, DuPont H L & Hornick R B. Fecal leukocytes in diarrheal illness. *Ann Intern Med* 76: 697 1972.
- 3 Looijers S, DeBoek M & Butzler J P. Campylobacter enteritis in Brussels. *Lancet* I 604 1978.
- 4 Maki M, Gronroos P & Vesikari T. In vitro invasiveness of *Yersinia enterocolitica* isolated from children with diarrhea. *J Infect Dis* in press.
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SHORT COMMUNICATION

EXOCRINE PANCREATIC INSUFFICIENCY SMALL INTESTINAL DYSFUNCTION AND PROTEIN INTOLERANCE

A Chance Occurrence or a Connection?

The coexistence of cystic fibrosis (CF) and coeliac disease (CD) is reported in eight children (for ref. see 4, 5) the odds against this occurring in the same child being 1:2 million to 1:5.9 million (4). In Sweden it would be around 1:3 million (incidence of CF 1:3000 (6) and of CD 1:1000 (2)). The coexistence of these two diseases is thought to be chance (5) although CF might predispose to the development of CD (4). In a 10-year period (with 33405 live births) we observed three children with exocrine pancreatic insufficiency and small intestinal dysfunction.

Patient 1. CF and CD. Boy only child of Jewish unrelated parent. At age 1 month he had bronchopneumonia with atelectasis. At 5, 7 and 8 months bronchitis. Gluten containing diet from age 4 months. From 6 months diarrhoea, failure to thrive and weight loss. At 8 months two pilocarpine iontophoresis gave a sweat sodium of 173 and 106 mmol/l. Duodenal juice had no trypsin and no amylase activities. No *Giardia lamblia*. Xylose and lactose tolerance tests abnormal. Intestinal biopsy showed a morphology in agreement with coeliac disease. Intestinal disaccharidases (cf. 3) very low (Table 1). He gained weight and dramatically improved on pancreatic extract and vitamins, physiotherapy, antibiotics and a gluten free diet. At 13 months intestinal biopsy showed marked morphological improvement (Table 1). Intestinal dipeptidase activities (cf. 3) against val-glu and ala-pro were extremely low (Table 1). Xylose test normal. Gluten challenge at 15 months gave diarrhoea and decreased xylose absorption. Two further gluten challenges in 4 and 5 years resulted in diarrhoea. Now at age 6.5 years he has normal weight and height.

Patient 2. CF and disaccharidase deficiency. Girl second child of unrelated parents and with a healthy brother. Recurrent respiratory infections from age 9 months, diarrhoea and no weight gain. Two pilocarpine iontophoresis at 1 month showed a sweat sodium of 100 and 97 mmol/l. No trypsin activity detected in duodenal juice. Xylose tolerance test normal, lactose tolerance test abnormal. Intestinal biopsy showed villous mucosa with unspaced mucous secretion in the crypts, normal intestinal dipeptidases, low disaccharidase activities (especially lactase

activity) (Table 1). No symptoms of disaccharide intolerance. Died at age 5.5 years.

Patient 3. Shwachman syndrome, food intolerance, decreased intestinal lactase, ala-pro and val-glu dipeptidase activities. Boy only child of unrelated parents. Admitted at age 4 months for infantile colic and eczema. He got diarrhoea and failed to thrive, had neutropenia and slight thrombocytopenia. On three occasions low or zero values of trypsin, amylase and lipase in duodenal juice. Pilocarpine iontophoresis $\times 3$ gave a sweat sodium of 17, 19 and 34 mmol/l. Xylose and lactose tolerance tests normal at age 4 months. Intestinal biopsy at 8 months showed a slightly damaged mucosa. Low val-glu and ala-pro dipeptidase activities and low lactase activity (Table 1). At 9 and 18 months urticaria after an egg and skin rash repeatedly after drinking milk. At 3 years he tolerated egg at 6 years milk. Height now at age 9 years 115 cm (-3 SD).

The small intestinal dysfunction reported in CF includes besides CD specific lactase deficiency (1, 8), reduced lactase activities (7, 8) and reduced uptake of phenylalanine and cycloleucine (8). In Shwachman syndrome (10) the morphology of the intestinal mucosa was reported normal (7, 10) or slightly changed (9, 10). Intestinal disaccharidases (7, 9) and enterokinase (7) normal.

Besides eczema observed in Shwachman syndrome (9, 10) our patient had allergic skin reactions after egg and milk, also low intestinal lactase, ala-pro and val-glu dipeptidase activities. These two dipeptidase activities were also proportionally further decreased in the second biopsy from the boy with CF+CD.

Abbreviations: CF=cystic fibrosis, CD=coeliac disease. Gly-leu=glycyl-L-leucine, Ala-glu=L-alanyl-L-glutamic acid, val-glu=L-valine-L-glutamic acid, glu-val=L-glutamyl-L-valine, ala-pro=L-alanyl-L-proline, ala-phe=L-alanyl-L-phenylalanine.

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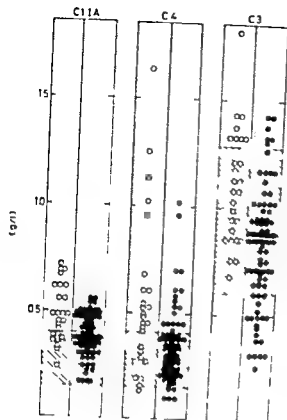


Fig. 2 Serum concentrations of C1-esterase inactivator and complement factors C3 and C4 in children with acute lymphoblastic leukemia before treatment or during bone marrow relapse (O) and in remission either on or off treatment (●). Shaded area=reference ranges.

figures for the complement factor C4 were for children with ALL before treatment or in relapse 0.54 g/l for children in remission either on or off treatment 0.33 g/l. For C3 the results were for children with ALL before treatment or in relapse 1.10 g/l and children in remission either on or off treatment 0.82 g/l. The pre-treatment or relapse values of C1A, C3 and C4 were significantly different from those following successful therapy ($p < 0.05$). The results from serial determinations of C1A in the five patients who relapsed are shown in Fig. 1. Only 2 of the patients had elevated C1A at the time of relapse and in all of them there were large fluctuations of the C1A serum levels. Similar observations were recorded for the other complement factors measured in the sera of these patients (data not shown). Subnormal serum lysozyme concentrations in patients with acute lymphoblastic leukemia have been regularly reported (4, 5, 7). Upon remission the lysozyme level usually returns to normal (7). According to Bratlid & Moe (4) serial determinations of lysozyme might be of value in leukemic

children under treatment to assess the effect of therapy and to discover early systematic relapses. Borch Mortensen et al (1, 2) reported increased C1A and C4 values in relapses of malignant diseases (including leukemia) and decreasing values during effective cytostatic treatment.

The results here presented, which are analogous to the fluctuations of serum ferritin in children with ALL (6) indicate that determinations of these serum components are of little assistance to detect or rule out relapse of ALL at an early stage.

ACKNOWLEDGEMENT

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REFERENCES

1. Astrup J, Colstrup H, Frandsen B. Complement C1 inactivator in the serum of patients with malignant diseases. *Acta Radiol Therap Phys Biol* 16: 39, 1977.
2. Borch Mortensen N, Osther K, Støwer I. C1-esterase inactivator and C4 in malignant disease. *Lett Cell* 11: 499, 1975.
3. Borgen J, Romslo I. Lysozyme determined in serum and urine by a simple nephelometric method. *Clin Chemistry* 23: 1999, 1977.
4. Bratlid D, Moe P. J. Serum lysozyme activity in children with acute leukemia. *Eur J Pediatr* 127: 63, 1978.
5. Finch S, Castro O, Lippman M, E. Donadon J, A. & Parille P. E. Lysozyme in leukopathic states. In Osseman E, F. Canfield H, E. & Beych S. (eds.) *Lysozyme*. Academic Press, New York, 1974, p. 335.
6. Koller M, E. Romslo I, Finne P, H. & Haneberg B. Serial determination of serum ferritin in children with acute lymphoblastic leukemia. Evaluation of its usefulness as a prognostic index. *Acta Paediatr Scand* 68: 93, 1979.
7. Moe P, J. Haneberg B, & Finne P, H. Serum lysozyme activity in children with hematological and malignant disorders. *Acta Paediatr Scand* 64: 530, 1975.

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CASE REPORT

HYPERBILIRUBINAEMIA AND IDIOPATHIC HYPOPHYSECTOMY IN THE NEWBORN PERIOD

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ABSTRACT Drop S L S Collier E and Guyda H J (Department of Paediatrics and Montreal Children's Hospital Research Institute Montreal Canada) Hyperbilirubinaemia and idiopathic hypopituitarism in the newborn period. *Acta Paediatr Scand* 68 277 1979. —Two infants with idiopathic panhypopituitarism presented with severe neonatal hypoglycaemia hepatomegaly and hyperbilirubinaemia (direct and indirect). Abnormal liver function tests returned to normal over a 5–8 month period. The growth rate in the absence of detectable growth hormone was 50% of normal during the first 6 months. The effect of growth hormone on somatomedin levels and growth rate during the first year of life in one of the infants is described.

KEY WORDS Hyperbilirubinaemia growth hormone somatomedin hypopituitarism

Congenital hypopituitarism due to a deficiency of hypothalamic hypophysiotropic hormones or aplasia of the pituitary gland is a well recognized syndrome consisting of severe and persistent hypoglycaemia and micropthalmos in neonates of normal birth weight and length (2, 11, 14, 16, 18). In addition liver dysfunction with clinical and histologic evidence of the neonatal hepatitis syndrome has been described in some of these infants (12). To underscore the importance of early detection and treatment of this syndrome we present two patients in whom the diagnosis of hypopituitarism was made within the first months of life. The linear growth rate in the absence of detectable growth hormone was 50% of normal during the first 6 months. The effect of GH treatment on somatomedin levels and growth rate during the first year of life in one of the infants is described.

CASE HISTORIES

Case 1
Patient J M was born after an uncomplicated term pregnancy with normal labor and delivery. The Apgar score was 8 at 1 min and 10 at 5 min. The birth weight

was 3.76 kg, length 50 cm, head circumference 36 cm. At 17 hours of age the baby had a generalized seizure. A serum glucose was 1.7 mmol/l, calcium 2.5 mmol/l. Six hours later another seizure occurred with a glucose level of 0.33 mmol/l. Physical examination disclosed an irritable neonatal baby with reddish blond hair. There was hepatomegaly (15 cm below the right costal margin) without splenomegaly. Glucose levels remained labile requiring glucose infusions of 8 mg/kg body weight/minute until the 13th day of life. Gradually normoglycaemia could be maintained with 4 hourly oral feedings.

On the second day of life the infant became jaundiced. Over the following 10 days she required 4 blood exchanges. The highest recorded indirect serum bilirubin level was 340 μ mol/l, highest direct 78 μ mol/l. The blood type of mother and baby was A positive. Peripheral blood smear from the parents and baby were normal. Direct and indirect Coombs tests were negative. Prior to the second exchange on the 4th day the serum LDH was 654 IU, SGOT 87 IU, alkaline phosphatase 5 IU. These liver function tests remained unchanged throughout the hospital stay. At three months of age the direct bilirubin was 59.85 μ mol/l, total 80.37 μ mol/l, SGOT 196 IU.

She received a 10 day course of antibiotics despite negative bacterial cultures of blood, urine and CSF. An IgM level prior to the first exchange was 0.76 g/l. Viral studies for rubella, CMV, RSV, parainfluenza 1, 2, 3 as well as studies for toxoplasma in infant and mother were negative. Fundoscopic examination of the eyes was normal. The hepatitis B antigen was negative. The sweat chloride, serum alpha 1 antitrypsin, alpha fetoprotein and G-6-PD levels were all normal. The urine was negative for ketones and reducing substances. Serum and urine amino acid chromatograms were normal. A roentgenogram of the

Table 1 Morphology, intestinal dipeptidases and disaccharidases in small intestinal biopsies (duodeno jejunal flexure) from the three patients

	1 CF+CD		2 CF	3 Shwachman syndrome
	On gluten	Without gluten		
Morphology				
Dissecting microscopy	Mucosa convoluted	Leaves+ridges	Finger+leaves	Leaves
Histology	Cuboidal and irregular epithelial cells. Flattening of crypts. Infiltration of plasma cells and leucocytes	Normal epithelial cells. Flattening of crypts. Infiltration of crypts. Infiltration of plasma cells	Normal epithelial cells. Slight elongation of crypts. Infiltration of crypts. Infiltration of plasma cells and leucocytes	Normal epithelial cells. Slight elongation of crypts. Slight infiltration of plasma cells and leucocytes
Intestinal dipeptidases (nmol/h)				
Gly leu (98-140)*	-	71	223	119-226*
Ala glu (20-60)	-	17	60	21-
Val glu (7.2-27)	-	<0.5	36	4.8-6.4
Glu val (7.2-41)	-	2.7	17	8.2-8.7
Ala pro (5.1-19)	-	0.8	13	1.0-1.9
Intestinal disaccharidases (u/g protein)				
Maltase (91-600)	16	-	24	160
Isomaltase (20-126)	7.8	-	9.5	54.4
Sucrase (23-166)	4.8	-	10.5	44.4
Trehalase (8.3-50)	0.5	-	2.2	5.3
Lactase (6.6-73)	0.8	-	1.1	5.8

* Controls range ($n=49$) (3) * Two biopsies from the same biopsy occasion

To conclude we found morphological and enzymatical abnormalities in intestinal mucosa in both CF and Shwachman syndrome favouring the hypothesis that exocrine pancreatic insufficiency predisposes to the development of small intestinal dysfunction in phenotypically high risk individuals.

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REFERENCES

- Antonowicz I Reddy V Khaw K T & Shwachman H Lactase deficiency in patients with cystic fibrosis *Pediatrics* 42 492 1968
- Berg N O & Lindberg T Incidence of coeliac disease and of transient gluten intolerance in a Swedish urban community *Acta Paediatr Scand* 68 1979 In press
- Dahlqvist A Lindberg T Meeuwisse G & Åkerström M Intestinal dipeptidases and disaccharidases in children with malabsorption *Acta Paediatr Scand* 59 621 1970
- Goodchild M C Nelson M & Anderson Ch M Cystic fibrosis and coeliac disease coexistence in children *Arch Dis Child* 48 654 1973
- Katz A J Falchuk M Z & Shwachman H The coexistence of cystic fibrosis and coeliac disease *Pediatrics* 57 715 1976
- Kollberg H Cystic fibrosis in Sweden Thesis *Acta Universitatis Upsalensis* 197 1974
- Lebenthal E Antonowicz I & Shwachman H Enterokinase and trypsin activities in pancreatic insufficiency and diseases of the small intestine *Gastroenterology* 70 508 1976
- Morrin C L Roy C C Laville R & Bonn A Small bowel dysfunction in patients with cystic fibrosis *J Pediatr* 83 213 1976
- Shmerling D H Prader A Hitzig W H Giedion A Hadorn B & Kuhn M The syndrome of exocrine pancreatic insufficiency neutropenia metaphyseal dysostosis and dwarfism *Helv Paediatr Acta* 24 547 1969
- Shwachman H Diamond L K Oki F A & Khaw K T The syndrome of pancreatic insufficiency and bone marrow dysfunction *J Pediatr* 65 635 1964

CASE REPORT

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KEY WORDS Hyperbilirubinaemia, growth hormone, somatomedin, hypopituitarism.

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CASE HISTORIES

Case 1

Patient J M was born after an uncomplicated term pregnancy with normal labor and delivery. The Apgar score was 8 at 1 min and 10 at 5 min. The birth weight

was 3.6 kg, length 40 cm, head circumference 36 cm. At 1st hours of age the baby had a generalized seizure. A serum glucose was 1.7 mmol/l, calcium 2.5 mmol/l. Six hours later another seizure occurred with a glucose level of 0.33 mmol/l. Physical examination disclosed an untable orontal baby with reddish blond hair. There was hepatomegaly (1.5 cm below the right costal margin) without splenomegaly. Glucose levels remained labile requiring glucose infusions of 8 mg/kg body weight/minute until the 15th day of life. Gradually normoglycaemia could be maintained with hourly oral feedings.

On the second day of life the infant became jaundiced. Over the following 10 days she required 4 blood exchanges. The highest recorded indirect serum bilirubin level was 340 µmol/l, highest direct 78.6 µmol/l. The blood type of mother and baby was A positive. Peripheral blood smear from the parents and baby were normal. Direct and indirect Coombs tests were negative. Prior to the second exchange on the 4th day the serum LDH was 654 IU, SGOT 87 IU, alkaline phosphatase 5 IU. These liver function tests remained unchanged throughout the hospital stay. At three months of age the direct bilirubin was 59.85 µmol/l, total 80.37 µmol/l, SGOT 196 IU.

She received a 10 day course of antibiotics despite negative bacterial cultures of blood, urine and CSF. An IgM level prior to the first exchange was 0.76 g/l. Viral studies for rubella, CMV, RSV, parainfluenza 1, 2, 3 as well as studies for toxoplasma in infant and mother were negative. Fundoscopic examination of the eyes was normal. The hepatitis B antigen was negative. The sweat chloride, serum alpha 1 antitrypsin, alpha fetoprotein and G-6-PD levels were all normal. The urine was negative for ketones and reducing substances. Serum and urine amino acid chromatograms were normal. A roentgenogram of the

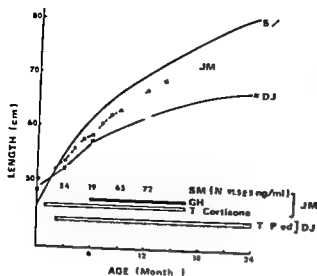


Fig. 1 Growth data in J.M. and D.J. is plotted using the 5th percentile from the National Center for Health Statistics Growth Chart as reference. The values for somatomedin radioreceptor activity (21) measured in plasma of J.M. are indicated by the numbers of the appropriate sampling times. Replacement therapy is indicated by the bars.

skull revealed no abnormalities. The bone age at 1 week of age was consistent with term maturity.

On the 25th day of life a needle biopsy of the liver was performed. It showed a few intrasinusoidal foci of extramedullary haematopoiesis. Portal tracts and perportal areas showed sparse infiltrate and round inflammatory cells occasionally arranged around bile canaliculi. Hyperplasia of Kupffer cells was also present.

On the 28th day of life cortisone acetate 5 mg t.i.d. was started followed 2 days later by 1 thyroxine 25 µg/d with an increment to 37.5 µg/d at two months of age. At 6 months of age GH treatment was initiated in a dose of 1 IU i.m. 3 times per week (provided by the Medical Research Council of Canada). The effects on growth rate and somatomedin levels are depicted in Fig. 1.

Case 2

Patient D.J., a Caucasian boy, was born of a 24-year-old G.III P.O. mother. At 12 weeks gestation the mother had a flu-like illness with vaginal spotting. Two weeks prior to delivery the mother had another flu-like illness with a diffuse maculopapular rash over her body and extremities including palms and soles as well as lymphadenopathy and joint pains. Ampicillin was given. Because of low urinary estradiol levels (4 and 4.7 mg/24 hr) delivery was per Caesarean section at 43 weeks gestation. Apgar score was 4 at 1 min, 7 at 6 min. The birth weight was 3315 g, length 48 cm, head circumference 37 cm. On physical examination a full fontanelle measuring 4×2 cm with separation of the sagittal sutures was noted. Liver and spleen were both palpable 2 cm below the costal margin. The testes were descended. The phallus was very small. At 2 hours of age a serum glucose level was 0.77 mmol/l. Recurrent hypoglycaemia was treated with intravenous glucose infusions up to 8 mg/kg body weight/minute for

the first 2 weeks of life. Two hourly oral feedings were introduced after 1 week.

On the 2nd day of life the patient developed a maculopapular rash lasting 10 days. Antibiotics were given. It was also noted to be jaundiced and required an exchange transfusion on day 2 (maximum serum bilirubin levels reached 311.2 µmol/l, direct 47.9 µmol/l). Indirect values decreased very gradually over the next two months while direct values climbed initially over the first 7 weeks to 133.4 µmol/l before returning to normal by 8 months of age. Liver enzymes prior to the exchange were SGOT 176 IU, SGPT 112 IU and LDH 1590 IU and highest levels were recorded at 2 months of age (SGOT 670 IU, SGPT 357 IU, LDH 1725 IU). Normal values were obtained at 8 months of age.

The blood type of mother and baby was A positive. Direct and indirect Coombs tests were negative. The peripheral blood smear was normal. Bacterial and viral cultures of throat, urine and stool remained negative. A serum IgM level (prior to exchange) was 0.08 g/l. Fundoscopic examination of the eyes was normal. Serology for mumps, rubella, CMC, herpes, varicella and toxoplasmosis in mother and baby remained negative. Serum alpha 1 antitrypsin and a G-6-PD level were normal. The hepatitis B antigen was negative. The urine was negative for ketones and reducing substances. A serum and urine amino acid chromatogram was normal.

At 8 weeks of age an open liver biopsy was performed. It showed moderately marked giant cell transformation with numerous bile thrombi in Zenker-fixed material compatible with the diagnosis of giant cell hepatitis. A roentgenogram of the skull showed a small flat pituitary fossa.

At 2 months of age prednisone in an initial dose of 3 mg t.i.d. and 1 thyroxine 50 µg/d were started. After 1 month of treatment the prednisone dose was decreased to 1 mg t.i.d. The patient remained prone to hypoglycaemia, especially during intercurrent illnesses and prolonged fasting. From 2½ years of age the patient received GH treatment according to the protocol of the Medical Research Council of Canada (9). Details of the endocrinologic data on both patients are given in Table 1.

DISCUSSION

The relationship between hypopituitarism and liver dysfunction with prolonged jaundice noted in several previous reports and reviewed by Herman (12) remains unclear. It has been hypothesized that the absence of hormones normally released by the pituitary might be responsible. Hypothyroidism secondary to TSH deficiency results in elevation of indirect bilirubin levels due to failure of conjugation in the absence of increased hemolysis. Corticosteroids have been reported to increase the enterohepatic circulation of bile salts (17).

Table 1 Endocrinologic evaluation

Determinations were made in plasma or serum during the first weeks of life before hormonal replacement

		J M	D J
	Range of normal values		
T4	77-141 mU/l	54	46
TSH	$<10 \times 10^{-3}$ int μ l	6	
Thyroidal I uptake	8-30%		17% at 24 hrs
Idem following TSH	5 U thyropar ⁴ 1 m increase of 10-20%		17.9% at 6 hrs
Cortisol 8 a.m.	33 nM/d	8.3	17.4% at 24 hrs
		17.4	
Cortisol following ACTH	6.5 U cortrosyn ⁶ 1 m -3 fold increase	- fold	
Insulin glucose ratio	<50	14.7	
		10.7	
<i>h</i> Provocative tests	Peak values		
Glucagon (0.1 mg i.m.)	Glucose	3.6 mU/l	2.09 mU/l
	GH (>5 ng/ml)	1 ng/ml	1 ng/ml
Arginine (400 mg/kg i.v. over 30 min)	GH (>4 ng/ml)	1 ng/ml	1 ng/ml
TRH (100 μ g i.v.)	GH (<5 ng/ml)	2.5 ng/ml	1 ng/ml
	PRL (>15 ng/ml)	<8 ng/ml	4.0 ng/ml
	TSH (>10 μ U/ml)	21×10^{-3} int μ l	12×10^{-3} int μ l

Range of normal for first week of life

Performed in J M at 7 months of age in D J at 5 1/2 years. thyroid medication was discontinued 2 weeks prior to testing. TSH, T4, GH, PRL and cortisol were determined by radioimmunoassay

However hyperbilirubinaemia and abnormal liver function tests persisted in these two infants after replacement therapy with cortisol and thyroxine was instituted a rise in the direct bilirubin characterized this period and an increase in liver enzymes. Studies in rats suggested that growth hormone can modulate bile acid synthesis (1). A single study in humans deficient in growth hormone revealed increased micellar phase bile acid in the jejunum following GH treatment. Whether the increase was due to the increased bile acid synthesis or secretion could not be determined from the design of the study. Decreased bile acid secretion could lead to cholestasis. It is of interest that bile acid concentrations normally increase with age and this could explain the spontaneous resolution of the abnormal liver function noted in these patients (17).

In both patients evidence of hypothyroidism and hypoadrenalism was present in the first weeks of life. Although no growth hormone could be detected upon stimulation with glucagon and arginine a growth rate of approxi-

mately 50% of normal during the first 4-5 months was observed.

The initiation of GH treatment in patient J M resulted in an increase of SM levels and a linear growth rate of up to 80% of normal in sharp contrast to the growth pattern of patient D J who did not receive GH treatment until age 2 1/2 (Fig. 1).

Somatomedin serum peptides with insulin like activities appear to play an important role in fetal and neonatal growth (4, 5, 7). Patients born with primary SM deficiency are retarded in length at birth (15, 20). A normal initial growth pattern generally seen in GH deficient infants (11, 12, 16) suggests that significant longitudinal growth can occur in the absence of GH. The finding of a SM level 50% of normal at 3 months of age in one of our patients is in full agreement with the hypothesis that not all SM production is dependent on stimulation by GH (4). There is experimental evidence that PRL and ovine placental lactogen can stimulate hepatic SM generation in the rat (6, 13) and the human (10). As well insulin an

important fetal growth factor was shown to sustain SM release in the perfused rat liver is effectively is GH (4)

In contrast to the report of Lovinger (18) basal PRL levels were low and the response to TRH was subnormal. However TSH release following TRH was normal in both patients suggesting hypothalamic dysfunction.

The two cases presented illustrate the importance of early diagnosis which in the male infant is facilitated by the apparent microphallus. Complete hormonal replacement therapy including GH is essential not only to relieve symptomatic hypoglycemia but also to prevent a permanent growth delay in infancy in which the response to GH is most favourable (9).

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REFERENCES

1. Behr W T, Behr M I & Semenuk G. The effect of pituitary and thyroid hormones on bile acid metabolism in the rat. *Metabolism* 15: 181, 1966.
2. Caccini E, Cicognini A, Piracoli P, Tissoni P, Bernini F, Sbardini M, Zuppoli F & Mizzanti L. Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus. Effect of GH therapy. *Helv Paediatr Acta* 31: 481, 1976.
3. Daughiday W H. Hormonal regulation of growth by somatomedin and other tissue growth factors. *Clin Endocrinol* 6: 117, 1977.
4. Daughiday W H, Phillips L M & Mueller M C. The effect of insulin and growth hormone on the release of somatomedin by the isolated rat liver. *J Lab Clin Med* 77: 803, 1976.
5. d'Ercole A J, Foyhee D B & Underwood L E. Somatomedin C receptor ontogeny and levels in porcine fetal and human cord serum. *J Clin Endocrinol Metab* 43: 1069, 1976.
6. Francis M J O & Hill D J. Prolactin stimulated production of somatomedin by rat liver. *Nature* 255: 167, 1975.
7. Gluckman P D & Brinsmerd M W. Somatomedin in cord blood: relationship to gestational age and birth size. *J Clin Endocrinol Metab* 43: 1378, 1976.
8. Guyda H J. Heterogeneity of hGH and prolactin secreted in vitro. Immunoassay and radioreceptor assay correlations. *J Clin Endocrinol Metab* 41: 953, 1975.

9. Guyda H J, Friesen H, Bailey J D, Lebed G & Beck J C. Medical Research Council of Canada. Therapeutic trial of human growth hormone first 5 years of therapy. *Can Med Assoc J* 117: 1301, 1975.
10. Guyda H J, Corvol M T, Rappaport R & Pomer B I. Radioreceptor assay of insulin like peptides in hum in plasma: growth hormone dependence and correlation with sulphation activity. Submitted for publication to *J Clin Endocrinol Metab*.
11. Henze G, Ruhl R & Werner E. Kombinierte hypophysäre Insuffizienz beim Säugling. *Monatsschr Kinderheilkd* 124: 394, 1976.
12. Hermi S P, Biggenstoss A H & Cloutier M D. Liver dysfunction and histologic abnormalities in neonatal hypopituitarism. *J Pediatr* 91: 897, 1977.
13. Hurley T W, d'Ercole A J, Hadwerner S, Underwood L F, Furlanetto R W & Fellows R F. Ovine placental lactogen induces Somatomedin: a possible role in fetal growth. *Endocrinology* 101: 1635, 1977.
14. Johnson J D, Hinsen R C, Albritton W L, Wertheimann U & Christensen R O. Hypoplasia of the anterior pituitary and neonatal hypoglycemia. *J Pediatr* 91: 634, 1977.
15. Iaron Z, Karp M, Pertzalan A, Kavi R, Keri R & Duron M. The syndrome of familial dwarfism and high plasma immunoreactive human growth hormone. In M. Pecile & E. Müller (eds.) *Growth and growth hormone*. Excerpta Medica Amsterdam, 1972, p. 458.
16. Lovinger R D, Kaplan S L & Grumbach M M. Congenital hypopituitarism associated with neonatal hypoglycemia and microphallus. Four cases secondary to hypothalamic hormone deficiencies. *J Pediatr* 97: 1171, 1975.
17. Poley J R, Smith J D, Thompson J B & Seeh J R. Improved micellar dispersal of dietary lipid bile acids during replacement therapy in growth hormone deficient children. *Pediatr Res* 12: 1186, 1977.
18. Sridhegi Nayud A & Senior H. A familial syndrome of isolated aplasia of the anterior pituitary. *J Pediatr* 84: 79, 1974.
19. Schiffrin A, Guyda H, Robitaille P & Pomer B. Increased plasma somatomedin reactivity in chronic renal failure as determined by acid gel filtration and radioreceptor assay. *J Clin Endocrinol Metab* 46: 511, 1978.
20. Vin den Brande J L, du Caju M V L, Vissel H K, A. Schopman W, Hackeng W H L & Degenhart H J. Primary somatomedin deficiency. Case report. *Arch Dis Child* 49: 297, 1974.

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CASE REPORT

CONGENITAL ASCITES DUE TO MESENTERIC VESSEL CONSTRICTION CAUSED BY MALROTATION OF THE INTESTINES

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ABSTRACT Hertel J & Volsted Pedersen P (Departments of Neonatology and Paediatric Surgery University Hospital Copenhagen Denmark) Congenital ascites due to mesenteric vessel constriction caused by malrotation of the intestines. *Acta Paediatr Scand* 68 281 1979.—A newborn premature girl with congenital non-chylous ascites is presented. The ascites recurred although laparocentesis was performed three times. The ascites was probably due to a superior mesenteric vein constriction caused by a malrotation of the intestines. After division of the Ladd bands no ascites recurred. Non-chylous congenital ascites may have a surgically treatable cause.

KEY WORDS Neonates non-chylous ascites malrotation of the intestines mesenteric vessel constriction portal hypertension

The relatively rare condition of congenital ascites has been described occurring in connection with obstructive uropathy, meconium peritonitis, hydrometrocolpos, fetal cytotomegalic inclusion disease, chylous ascites, pancreatitis, polycystic kidneys or liver, Wilms tumor, neuroblastoma, organomegaly and anterior meningocele (4).

We present a case of congenital ascites which was possibly caused by constriction of the superior mesenteric vessels and disappeared after operation for malrotation of the intestines.

CASE STORY

A female infant was delivered after 37 weeks of gestation. The pregnancy had been complicated by bleeding in the first month and by mild pre-eclampsia which was treated with diuretics and sedatives during the last month of the pregnancy. The mother was 34 years old and this was her first pregnancy.

Five days before delivery an ultrasound scan disclosed fetal ascites and she was admitted to this hospital where labour started shortly after but was stopped by Utopar®. Betamethasone was also given. 5 days later labour recommenced but this time could not be stopped. Delivery was

uncomplicated and the Apgar scores were 7 after 1 min and 9 after 5 min. Birthweight was 1910 g and the length 41 cm.

The infant had a large abdomen with a cone shaped thorax and very thin extremities with a mild equinovarus malformation of both feet. The external genitalia were normal. Voiding was spontaneous and sterile at culture. Because of respiratory distress a laparocentesis was performed and 750 ml of clear yellow sterile fluid was obtained. The abdominal wall was now very lax and no intra abdominal abnormalities could be palpated.

Oral feeding with Pregestimil® (a formula containing medium chain triglycerides, glucose and hydrolysed protein) was instituted together with intravenous glucose-saline over the next few days. Her oral intake varied. During this time she passed loose stools daily without blood and never showed any signs of ileus. At 10 days of age she could only take very little orally and the weight had decreased to 1490 g. Parenteral nutrition was started.

Although diuretics and albumin were given the abdominal circumference increased and laparocentesis was performed when she was 3 and 4 weeks old yielding 80 and 180 ml of clear fluid respectively. The ascites contained no fat, very few cells and the concentrations of sodium and potassium were equal to those of serum. In the first laparocentesis the protein concentration was 2.95 g/l. In the second laparocentesis the albumin concentration was 4.78 mmol/l while the serum concentration was 31.5 mmol/l. A test for amylase was not performed on the ascitic fluid. An intravenous pyelogram was judged as normal as were the ureters and the bladder. A barium

follow through of the gastrointestinal tract disclosed a malrotation of the intestines without any signs of stenosis. An angiocardiology showed the inferior caval vein to be normal and an urography disclosed a slightly enlarged spleen and a normal liver.

At 1 month of age an exploratory laparotomy was performed because of recurrent ascites, continuous loss of weight and failure to respond to medical treatment.

The intestines were found dilated and edematous and they were covered with fibrous adhesions. The peritoneal fluid was sterile in culture. The intestinal tract was malrotated with the third part of the duodenum in front of the superior mesenteric vessels which were slightly compressed by the duodenum. It was noted that a slight traction on the upper part of the small intestine caused ischemia of the most distal part of the duodenum and the upper part of the jejunum. The pancreas, the liver, the kidneys, the bladder and the internal genitalia all looked normal but the spleen was slightly enlarged. The Ladd bands were divided and after that no vascular insufficiency was observed.

After the operation intravenous nutrition was continued and oral feeding was restarted after 3 days. 2 weeks later she was tolerating oral feeding and was gaining weight. She was discharged from the neonatal department when 3 months of age weighing 2480 g. At follow up she continued to thrive and no signs of ascites were seen. At 18 months of age an intravenous pyelogram and a cystourography was performed because of pyuria and disclosed bilateral vesico ureteral reflux and a slight dilatation of the right ureter.

DISCUSSION

In an experimental study on dogs Witte et al (9) in 1969 showed that ascites developed after extrahepatic portal hypertension had been produced by a shunt between the aorta and the portal vein combined with restriction to the trans hepatic blood flow. Intestinal edema also developed. The protein content of the ascites was low and almost exclusively albumin. This was in contrast to experimentally induced chronic constriction of the inferior caval vein where the ascites produced was of high protein content and contained a large globulin fraction.

Chylous ascites is characterized by milky ascites especially after meals containing fat. The etiology is unknown in most cases but it is often a self limiting disease. The treatment consists of supportive measures with intravenous nutrition and an oral diet containing short chain triglycerides and high protein (7, 8). The long term prognosis is usually good

though Sanchez et al (7) noted central nervous system aberrations in 3 of 11 infants with chylous ascites.

Non chylous congenital ascites with lower urinary tract obstruction has been known for long time (5). About 50% of all cases of congenital ascites have accompanying urinary tract abnormalities (6). The ascites may be due to leakage or transudation of urine from the bladder, the ureters or the kidneys. The protein content in the ascites is usually low (3, 6).

A few cases of ascites due to pancreatic fluid leakage into the peritoneal cavity have been reported (2). Pancreatitis is an unusual condition in infancy, most often caused by abdominal trauma. Other possible causes are hereditary pancreatitis, measles infection, virus infection, steroid medication (1), hyperlipidemia and abnormalities of the pancreatic ducts (4). In our patient the pancreas was found to be normal at operation. There were no signs of major genito urinary abnormalities in the neonatal period. Urine was passed normally and was normal except for a slight proteinuria. She later developed bilateral reflux and dilatation of the right ureter.

The ascites in our patient was never chylous. The first laparocentesis was clear but was performed before any food had been given. The next two laparocenteses were performed after breast milk had been given and the ascites would thus have been milky if it had been of the chylous type.

The observations during operation of superior mesenteric vessel constriction and ischemia caused by traction on the Ladd bands and on the intestines are consistent with ascites due to extrahepatic portal hypertension combined with restriction of the transhepatic blood flow. The protein content of the fluid and the normal appearance of the liver along with slight enlargement of the spleen support this hypothesis. After the constriction was released no ascites was seen.

Non chylous congenital ascites may have a surgically treatable cause and an exploratory

laparotomy should be performed if medical supportive treatment and repeated laparocenteses are not successful

REFERENCES

- 1 Baar H E & Wolff O H Pancreatic necrosis in cortisone treated children *Lancet* **I** 81 1957
- 2 Coupland G Pancreatic ascites. A rare complication of pancreatitis in childhood *Aust NZ J Surg* **40** 5 1971
- 3 France N E & Back E H Neonatal ascites as associated with urethral obstruction *Arch Dis Child* **29** 565 1954
- 4 Gryboski J *Gastrointestinal Problems in the Infant* p 183 Saunders Philadelphia 1975
- 5 Marx R & Dale W A Neonatal ascites and obstructive uropathy *Pediatrics* **77** 9 1961
- 6 Radman H M Dystocia due to fetal abdominal enlargement *Obstet Gynecol* **19** 481 196
- 7 Sanchez R E Mahour G H Brennan L P & Wooley M M Chylous ascites in children *Surgery* **69** 183 1971
- 8 Sturmer jr F C Infantile chylous ascites *Am Surg* **31** 81 1965
- 9 Witte C L Chung Y C Witte M H Sterle O F & Cole W R Observations on the origin of ascites from experimental extrahepatic portal congestion *Ann Surg* **170** 100 1969

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- 2 Coupland G Pancreatic ascites. A rare complication of pancreatitis in childhood *Aust NZ J Surg* **40** 5 1971
- 3 France N III & Back E H Neonatal ascites associated with urethral obstruction *Arch Dis Child* **29** 465 1954
- 4 Gryboski J *Gastrointestinal Problems in the Infant* p 183 Saunders Philadelphia 1975
- 5 Marx A & Dale W A Neonatal ascites and obstructive uropathy *Pediatrics* **27** 9 1961
- 6 Radman H M Dystocia due to fetal abdominal enlargement *Obstet Gynecol* **19** 481 1967
- 7 Sanchez R III Mahour G H Brennan L P & Wooley M M Chylous ascites in children *Surgery* **69** 183 1971
- 8 Sturmer jr F C Infantile chylous ascites *Am Surg* **31** 281 1965
- 9 Witte C L Chung Y C Witte M H Sterle D F & Cole W III Observations on the origin of ascites from experimental extrahepatic portal congestion *Ann Surg* **170** 100 1969

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CASE REPORT

HYPOGLYCAEMIA AND CONGENITAL ADRENAL HYPERPLASIA

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ABSTRACT Gemelli M De Luca F and Barberio G (2nd Department of Paediatrics University Hospital Messina Italy) Hypoglycaemia and congenital adrenal hyperplasia. *Acta Paediatr Scand* 68 285 1979.—We report the case history of a child with congenital adrenal hyperplasia which was complicated by recurrent hypoglycaemic episodes during common infections. There are few reports in literature on the association of hypoglycaemia and congenital adrenal hyperplasia. In accordance with others we believe that hypoglycaemic attacks in adrenal hyperplasia are perhaps more frequent than recorded in literature.

KEY WORDS Hypoglycaemia adrenal hyperplasia

As hypoglycaemia is not a well recognized complication of 21 hydroxylase deficiency we consider it useful to report the case history of a child with a salt losing congenital adrenal hyperplasia and frequent hypoglycaemic attacks.

CASE REPORT

D.A. was born in Messina to parents who are first cousins. The mother bore four sons. The first two died during the first month of life because of uncontrollable vomiting and diarrhoea, the third is well and the fourth was admitted to our Clinic in the first hours of life with respiratory distress. During the hospitalization he had seizure, vomiting, dehydration and skin pigmentation. The clinical and laboratory data (plasma Na 94 mmol/l, K 7.7 mmol/l, cortisol 30 µg/l, 17 hydroxyprogesterone 84 µg/l, urinary 17 ketosteroids 9.1 mg/4 h) suggested the diagnosis of salt losing congenital adrenal hyperplasia. The symptoms rapidly disappeared and the biochemical parameters returned to normal after treatment with 9 α fluorohydrocortisone (10.1 mg/day) and cortisone acetate (40 mg/m²/day) was begun.

Since then his growth has been good and the plasma electrolytes and 17 hydroxyprogesterone, urinary 17 ketosteroids and bone age have always been normal.

When he was aged 7 months during an attack of enteritis the patient showed changes of mood, drowsiness, perspiration and had generalized seizures. He was immediately hospitalized and underwent laboratory

investigations. The plasma electrolytes were normal but the blood glucose was 1.11 mmol/l. The hypoglycaemia and convulsions were rapidly controlled by intravenous glucose. During this hospitalization an intravenous insulin tolerance test was performed (4 U/m). This provoked marked hypoglycaemia (1.16 mmol/l) with pallor and sweating but the glucose recovery index was only slightly decreased (170%). This test revealed normal plasma growth hormone (maximum 15 ng/l) and glucagon (maximum 90 ng/l) responses and a normal increase of urinary epinephrine (680%) in the three hours after the insulin injection while plasma cortisol remained substantially unchanged (from 38 to 41 µg/l).

Since then following our suggestions the child's parents have given him hydrocortisone by injection and sweetened drinks whenever they have observed any change of mood, drowsiness or increased sweating which have generally occurred during intercurrent infections.

In spite of these precautions during the last three years the child has six times developed more or less serious neurological symptoms such as drowsiness, convulsions and coma in association with common infections.

On these occasions marked hypoglycaemia (0.55–1.18 mmol/l) was present while the electrolytes were always normal. Glucose infusion provoked rapid resolution of the symptoms. The seizures persisted several hours on only one occasion when the child was taken to another hospital. The doctor on duty probably not knowing the risk of hypoglycaemia merely injected him with phenobarbital which was ineffective in controlling the convulsions. It is notable that all the electroencephalograms to date have always been normal.

DISCUSSION

Hypoglycaemia has been very rarely reported in association with 21 hydroxylase deficiency (4-6-9) which is not generally listed among the common causes of hypoglycaemia (1-5). Hypoglycaemic attacks occurred in only 2 out of 140 cases of adrenal hyperplasia followed up by Wilkins (8). According to Sauls & Ulstrom (6) congenital adrenal hyperplasia accounts for 4% of cases of spontaneous hypoglycaemia. Job et al (2) reviewing the acute symptoms occurring over 8 years in 11 children with congenital 21 hydroxylase deficiency report no hypoglycaemic episodes. In a review by Zupping (9) the occurrence of hypoglycaemia in children with adrenal hyperplasia is 1/40. Limbeck & Kelley (3) confirm the extremely infrequent reports of this complication which they expected to be more frequent considering the importance of cortisol in the maintenance of blood glucose. Mackinnon & Grant (4) in 1977 reported two cases of 21 hydroxylase deficiency with hypoglycaemic seizures and emphasized that the risk of hypoglycaemia in this condition needs more accurate evaluation. They suggested that this complication is perhaps more frequent than recorded in literature since it may at times be misinterpreted. Their hypothesis seems to be confirmed by the history of our patient in whom hypoglycaemic convulsions occurred fairly frequently as a consequence of the stress of infection and in whom they were once wrongly attributed to epilepsy.

REFERENCES

- 1 Ichiba Y & Bargman G I. Hypoglycaemia. In I Gardner (ed) *Endocrine and genetic disease childhood and adolescence*. W. B. Saunders & Philadelphia 1975 p 931.
- 2 Job J C, Canlorbe P, Lambert J, Courroux & Rossier A. Les accidents aigus au cours traitement de l'hyperplasie surrénale congénitale saine par défaut de 21 hydroxylase. *Ann Pediatr* 476 1970.
- 3 Limbeck G & Kelley V. Congenital adrenal hyperplasia. In V C Kelley (ed) *Metabolic endocrine genetic disorders of children*. Hagerstown (Maryl) 1974 p 765.
- 4 Mackinnon J & Grant D B. Hypoglycaemia in congenital adrenal hyperplasia. *Arch Dis Child* 57 1977.
- 5 Pagliaro A S, Karl I E, Haymond M & his D M. Hypoglycaemia in infancy and childhood. *Pediatr* 87 365 1973.
- 6 Sauls H S Jr & Ulstrom R A. Hypoglycaemia. In V C Kelley (ed) *Brenneman's Practice of pediatrics*. W. F. Prior Co. Hagerstown Maryland 1 vol 1.
- 7 White F P & Sutton L E. Adrenogenital syndrome with associated episodes of hypoglycaemia. *J Clin Endocrinol* 11 1395 1951.
- 8 Wilkins L. *The diagnosis and treatment of endocrine disorders in childhood and adolescence*. Thor Springfield Ill 1969 3rd ed p 546.
- 9 Zupping H A. Hypoglycaemia caused by diminished gluconeogenesis. In H A Zupping (ed) *Hypoglycaemia in childhood. Evaluation of diagnostic procedures*. S. Karger AG Basel Switzerland 1975 p 107.

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CASE REPORT

STRESS POLYCYTHAEMIA AND PERIPHERAL FACIAL PALSY
COMPLICATIONS OF SEVERE HYPERTENSION

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From the Department of Paediatrics University of Illinois Hospital Chicago USA

ABSTRACT Zeis P M Rao S John E G and Aschinberg L C (Department of Paediatrics University of Illinois Hospital Chicago USA) Stress polycythaemia and peripheral facial palsy complications of severe hypertension. *Acta Paediatr Scand* 68: 287-289, 1979.—An 11 month old boy had an episode of generalized convulsions followed by a right peripheral facial palsy which resolved gradually within 3 weeks. Three months later he had another similar episode of convulsions followed by a left peripheral facial palsy. On both occasions it was found that he had polycythaemia. A careful physical examination discovered that the child had severe hypertension. Extensive laboratory investigations did not reveal a cause for his hypertension. Haematologic investigations showed that the polycythaemia was due to a contracted plasma volume as a result of the hypertension. The peripheral facial palsy most probably was due to a blood clot in the facial canal below the origin of the nerve to the stapedius. His audiograms were normal and lacrimation preserved. Control of the hypertension resulted in resolution of the facial palsy within 4 weeks and normal haematocrit readings within 6 weeks. It should be stressed that every patient with peripheral facial palsy should be examined for hypertension.

KEY WORDS Hypertension polycythaemia facial palsy

Polycythaemia has been reported in adults as an infrequent complication of hypertension (2). Another complication of severe hypertension more common in adults than in the paediatric age group is peripheral facial palsy (4, 5).

We report a 14 month old boy with polycythaemia and peripheral facial palsy who was found to have severe hypertension.

CASE REPORT

A 14 month-old boy was admitted to the University of Illinois Hospital with the diagnosis of malignant hypertension. The boy was well until the age of 11 months when he had an episode of generalized convulsions followed by a right peripheral facial palsy for which he was admitted and investigated in a suburban hospital. The only positive finding was polycythaemia. The facial palsy resolved gradually within 3 weeks and he was discharged from the hospital. He was readmitted 3 months later into the same hospital following a new episode of generalized convulsions with a left peripheral facial palsy. The patient was investigated again; the polycythaemia was reconfirmed but it was also found that he had hypertension

with blood pressure readings ranging from 180/110 to 200/100 mmHg. He was then transferred to the University of Illinois Hospital.

On examination the left facial palsy was obvious (Fig 1). The blood pressure checked repeatedly ranged between the levels mentioned above.

Extensive investigations were carried out for his hypertension according to the schedule we practice in our department (1). The following investigations were performed: Detailed physical examination and fundoscopy; urinalysis and urine culture; chest roentgenogram and electrocardiogram; skull roentgenogram; electroencephalogram and computerized axial tomography; Urine concentration determinations and Addison count. Complete blood cell count; sodium, potassium, chloride, glucose, BUN, creatinine, β_2 C, PH, bicarbonate, cholesterol and triglycerides; Lipoprotein electrophoresis and serum cortisol analysis; Iodohippurate and technetium renal scans. Twenty four hours urine collection for creatinine and urea clearance; total protein excretion; 17 ketosteroid and 17 hydroxycorticosteroid determinations. Collection of urine for catecholamine quantification. Peripheral-vein renin activity determination; measurement of serum aldosterone, electrolyte levels and aldosterone excretion rates in a 24 hours urine collection. Contracted peripheral vein renin activity and selective renal vein renin activity were determined. A percutaneous renal biopsy was performed.

DISCUSSION

Hypoglycemia has been very rarely reported in association with 21-hydroxylase deficiency (4, 6-9) which is not generally listed among the common causes of hypoglycemia (1, 5). Hypoglycemic attacks occurred in only 2 out of 140 cases of adrenal hyperplasia followed up by Wilkins (8). According to Sauls & Ulstrom (6) congenital adrenal hyperplasia accounts for 4% of cases of spontaneous hypoglycemia. Job et al (2) reviewing the acute symptoms occurring over 6 years in 11 children with congenital 21-hydroxylase deficiency report no hypoglycemic episodes. In a review by Zupping (9) the occurrence of hypoglycemia in children with adrenal hyperplasia is 1/40. Limbeck & Kelley (3) confirm the extremely infrequent reports of this complication which they expected to be more frequent considering the importance of cortisol in the maintenance of blood glucose. Mackinnon & Grant (4) in 1977 reported two cases of 21-hydroxylase deficiency with hypoglycemic seizures and emphasized that the risk of hypoglycemia in this condition needs more accurate evaluation. They suggested that this complication is perhaps more frequent than recorded in literature since it may at times be misinterpreted. Their hypothesis seems to be confirmed by the history of our patient in whom hypoglycemic convulsions occurred fairly frequently as a consequence of the stress of infection and in whom they were once wrongly attributed to epilepsy.

REFERENCES

- 1 Ichiba Y & Bargman G I. Hypoglycemia. In L I Gardner (ed) *Endocrine and genetic diseases of childhood and adolescence*. W B Saunders & Co Philadelphia 1975 p 911.
- 2 Job J C, Canlorbe P, Lambert J, Courouble P & Rossier A. Les accidents aigus au cours du traitement de l'hyperplasie surrénale congénitale vincente par défaut de 21-hydroxylase. *Ann Pediatr* 17: 476, 1970.
- 3 Limbeck G & Kelley V. Congenital adrenal hyperplasia. In V C Kelley (ed) *Metabolic endocrine and genetic disorders of children*. Hagerstown (Maryland) 1974 p 265.
- 4 Mackinnon J & Grant D B. Hypoglycaemia in congenital adrenal hyperplasia. *Arch Dis Child* 52: 491, 1977.
- 5 Pighira A S, Karl I E, Haymond M & Kipnis D M. Hypoglycemia in infancy and childhood. *J Pediatr* 82: 365, 1973.
- 6 Sauls H S Jr & Ulstrom R A. Hypoglycemia. In V C Kelley (ed) *Brenneman's Practice of pediatrics*. W F Prior Co. Hagerstown Maryland 1966 vol 1.
- 7 White F P & Sutton L E. Adrenogenital syndrome with associated episodes of hypoglycemia. *J Clin Endocrinol* 11: 1395, 1951.
- 8 Wilkins L. *The diagnosis and treatment of endocrine disorders in childhood and adolescence*. Thomas Springfield Ill 1965 3rd ed p 546.
- 9 Zupping H A. Hypoglycemia caused by diminished gluconeogenesis. In H A Zupping (ed) *Hypoglycemia in childhood. Evaluation of diagnostic procedures*. S Karger A G. Basel Switzerland 1975 p 103.

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CASE REPORT

STRESS POLYCYTHAEMIA AND PERIPHERAL FACIAL PALSY COMPLICATIONS OF SEVERE HYPERTENSION

P. M. ZEIS, S. RAO, E. G. JOHN and L. C. ASCHINBERG

From the Department of Pediatrics, University of Illinois Hospital, Chicago, USA

ABSTRACT Zeng P M, Rao S, John E U and Aschenberg L C (Department of Paediatrics, University of Illinois Hospital, Chicago, USA). Stress polycythaemia and peripheral facial palsy: complications of severe hypertension. *Acta Paediatr Scand* 68: 287, 1979.—An 11 month-old boy had an episode of generalized convulsions followed by a right peripheral facial palsy which resolved gradually within 3 weeks. Three months later he had another similar episode of convulsions followed by a left peripheral facial palsy. On both occasions it was found that he had polycythaemia. A careful physical examination discovered that the child had severe hypertension. Extensive laboratory investigations did not reveal a cause for his hypertension. Haematologic investigations showed that the polycythaemia was due to a contracted plasma volume as a result of the hypertension. The peripheral facial palsy most probably was due to a blood clot in the facial canal below the origin of the nerve to m. stapedius as audiograms were normal and lacrimation preserved. Control of the hypertension resulted in resolution of the facial palsy within 4 weeks and normal haematocrit readings within 6 weeks. It should be stressed that every patient with peripheral facial palsy should be examined for hypertension.

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CASE REPORT

A 14 month-old boy was admitted to the University of Illinois Hospital with the diagnosis of malignant hypertension. The boy was well until the age of 11 months when he had an episode of generalized convulsions followed by a right peripheral facial palsy for which he was admitted and investigated in a suburban hospital. The only positive finding was polycythaemia. The facial palsy resolved gradually within 3 weeks and he was discharged from the hospital. He was readmitted 3 months later into the same hospital following a new episode of generalized convulsions with a left peripheral facial palsy. The patient was investigated again the polycythaemia was recon-

firmed but it was also found that he had hypertension with blood pressure readings ranging from 180/110 to 220/110 mmHg. He was then transferred to the University of Illinois Hospital.

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Extensive investigations were carried out for his hypertension according to the schedule we practice in our department (I). The following investigations were performed: Detailed physical examination and laboratory analysis and urine culture chest x-ray electrocardiogram skull roentgenogram in the cervical cephalogram and computerized axial tomography renal concentration determinations and A&C's arterial blood cell count sodium potassium chloride water BUN creatinine PC PH bicarbonate water and triglycerides Lipoprotein electrophoresis and cortisol analysis iodine hippuric acid scans Twenty four hour urine collection for urea and urea clearance test Ig serum cholesterol and 17 hydroxycorticosteroids and metabolites of urine for catecholamine excretion 24 hour urinary renin activity determined by radioimmunoassay of doxaprine electrode life span studies and survival rates in a 24 hours test interval for survival venous renin activity determined by radioimmunoassay were determined.

CASE REPORT

THE ACIDIFICATION DEFECT IN THE SYNDROME OF RENAL TUBULAR ACIDOSIS WITH NERVE DEAFNESS

H. SIMÓN, B. ORIVE, I. ZAMORA and S. MENDIZABAL

From the Paediatric Nephrology Unit, Hospital Infantil La Fe, Valencia, Spain

ABSTRACT Simón J, Orive B, Zamora I and Mendizabal S (Paediatric Nephrology Unit, Hospital Infantil La Fe, Valencia, Spain). The acidification defect in the syndrome of renal tubular acidosis with nerve deafness. *Acta Paediatr Scand* 1979; 68: 291-295. An 8-year-old boy with renal tubular acidosis and nerve deafness has been followed for seven years. Repeated studies of his renal acidification defect showed that until the age of six years the tubular defect was mixed proximal and distal (type 1/2 hybrid). After that age the defect of proximal acidification disappeared and the patient only presented a distal renal tubular acidosis type 1. When this is associated with nerve deafness it is considered a distinct nosological entity.

KEY WORDS Renal tubular acidosis, hereditary kidney disease, nerve deafness, nephrocalcinosis, acid base balance.

The syndrome of renal tubular acidosis (RTA) and nerve deafness has aroused interest over other associations of RTA and genetic diseases in the last decade. The study of 18 cases described (3, 6, 13, 20) or referred (5, 12, 14, 17) suggest that we are dealing with a differentiated nosological entity with recessive autosomal genetic transmission. The study of the tubular acidification defect has been documented only in three of the published cases (6, 14). However, all reports classify the RTA as distal or type 1. The retrospective study of the cases published by Cohen (19) has raised the doubt that one of them have a distal RTA with bicarbonate wasting or RTA type 3.

This paper presents the follow up of a one-year-old male affected by the syndrome of RTA and nerve deafness. The renal acidification defect was initially diagnosed as a mixed RTA or type 1/2 hybrid. At the age of seven the proximal defect disappeared while the distal RTA continues. This evolutive aspect of the tubulopathy, not referred to in the RTA

nerve deafness syndrome, could be related to studies of Saphira (18) on carbonic anhydrase activity.

CASE REPORT

J. P. C. male consanguineous, born at term after a normal pregnancy and delivery. Birth weight 3600 g. At 13 months of age he was admitted to the hospital for persistent vomiting and failure to thrive (weight and height below the 3rd percentile). Blood studies were: pH 7.13, P_{CO_2} 4 kPa, sodium 141 mmol/l, potassium 4.5 mmol/l, chloride 114 mmol/l, calcium 2.24 mmol/l, phosphorus 1.6 mmol/l, creatinine 44 μ mol/l. Calciuria was always higher than 0.25 mmol/kg/d. Urinary pH were between 7 and 8. Early morning urinary specific gravity after subcutaneous pitressin 10 IU. In the urine protein and glucose were absent. Blood and urine aminoacid screening were normal. Radiological examination showed bilateral nephrocalcinosis. A diagnosis of renal tubular acidosis was made and 6.5 mmol/kg/d of oral sodium bicarbonate were needed to maintain normal acid base status. At 4 years of age because of speech delay hearing was tested. The audiogram showed bilateral nerve deafness (Fig. 1).

Family study. The double consanguinity of the parents is shown in Fig. 1. Neither deafness nor nephrolithiasis was detected in the family history. In both parents and brother urinary acidification, calciuria and urinary concentration were investigated. All studies were normal.

Table 1 Ammonium chloride loading test

	Patient age (years)	Plasma		Urine (micromol/min/1.73 m ²)				$\frac{C_{CO_2}}{C_i} \times 100$
		pH	HCO ⁻ (mmol/l)	pH	TA	UV _{H₂}	UV _{HCO₃}	UV _{H₂}
Study 1	3 10/12	7.76	11	7.77			53.6	3.8
	5 2/12	7.76	11.7	7.70				
Study	7 7/12	7.71	10.8	7.0	4.6	79.7	5.0	3.3
Normal values								
Mean (SD)					57 (33-71)	73 (46-100)	0	1.5

Normal values according to Edelmann et al (7)

Renal study of acid base equilibrium Tests of acidification were performed at ages of 3 10/12 years, 5 2/12 years and 7 7/12 years. They showed a consistent urinary acidification defect with a urinary pH always higher than 7 even at maximal acidemia (Table 1). Reabsorption of bicarbonate was tested at ages of 5 2/12 years (study 1) and 7 7/12 years (study 2) as shown in Fig. 4. Both studies showed a constant plateau of bicarbonate excretion under 4% of the filtrate from levels of maximum acidemia to plasma bicarbonate concentrations of 15 mmol/l. In study 1 (open signs) from this plasma bicarbonate concentration excretion abruptly increased reaching 27% of the filtrate at normal plasma levels. In study 2 (closed signs) the plateau of bicarbonate excretion was consistently below 4% of the filtrate from levels of maximum acidemia to normal plasma concentrations of 20 mmol/l.

Tubular reabsorption of sodium Tubular reabsorption of sodium during the hypotonic

saline diuresis was checked at ages of 5 10/12 years (study 1) and 7 7/12 years (study 2). The data in Table 3 correspond to the period of minimum urinary osmolality (U_m) and maximum free water clearance (C_{H_2O}) when the activity of the antidiuretic hormone is minimal or absent. $C_{H_2O} + C_{Na}$ could at this moment be an approximate indication of the amount of sodium delivery to distal tubule escaping proximal reabsorption. Urinary volume represents the filtrate volume that reaches Henle's loop. In study 1 a marked increase of $C_{H_2O} + C_{Na}$ (22 ml/100 ml GFR) was noted coinciding with the proximal loss of bicarbonate. In study 2 the $C_{H_2O} + C_{Na}$ decreased to 14 ml/100 ml GFR simultaneously to the observed normalization in the proximal reabsorption of bicarbonate.

The data outlined of C_{in} (137 and 128 ml/min/1.73 m²), RTP (84% and 91%) and $Tm_{P_{CO_2}}$ (3.4 and 3.9 mg/100 ml GFR) were obtained prior to expansion with hypotonic saline infusion.

Table 2 Bicarbonate titration studies

	Patient age (years)	Plasma		$\frac{UV}{C} \times 100$	$\frac{C_{CO_2}}{C_i} \times 100$
		pH	HCO (mmol/l)	(micromol/100 ml GFR)	
Study I	5 1/12	7.6	11.7	46.3	3.8
		7.44	0.0	538.6	6.9
Study	7 7/12	7.71	10.8	35.4	3.3
		7.46	0.3	97.8	4.8

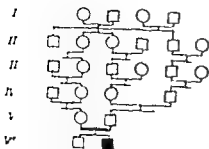


Fig. 1 The pedigree of the kindred

METHODS

Glomerular filtration rate (GFR) was estimated by inulin clearance (C_i) according to the standard method. Tubular reabsorption of phosphorus (RTP) and its maximum tubular transport (Tm_{RTP}) were calculated according to Björk's nomogram (1).

Urinary excretion of hydrochloric acid. An oral dose of 6 g/m² SA of ammonium chloride was administered according to Edelmann et al (7). During the next five hours plasma bicarbonate, urinary pH, titratable acidity (TA), ammonium excretion (U_{NH_4}) and net acid excretion (U_{NA}) were determined.

Bicarbonate titration studies. Assessment of tubular reabsorption of bicarbonate was performed according to the procedure described by Edelmann et al (8). Special care was taken to minimize extracellular volume expansion, keeping the bicarbonate infusion at a constant rate lower than 1 ml/min/m² SA.

Renal control of sodium and dilution ability. After an oral water overload in the preceding hour (70 ml/kg b.w.) a 0.45% hypotonic saline infusion was begun (2). At a rate of 4 ml/min/m² SA plus the urinary volume, the total amount administered was 2000 ml/1.73 m.

Analytic procedure. Inulin concentration was determined by the anthrone method of Davidson & Sickner (4), phosphorus according to the Fiske & Subbarow method (9). Blood pH and carbon dioxide content were measured in a Radiometer ABL 1. The urines were processed immediately after collection and the carbon dioxide was

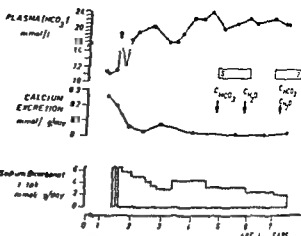


Fig. 3 Clinical course. Biochemical features as influenced by alkali therapy.

determined by a Natelson microassayometer. Urinary bicarbonate concentration was calculated from urinary pH and carbon dioxide content by the Henderson-Hasselbalch equation: $pH = 6.33 - 0.41(\log [Na^+] + (H^+))$ (10). Titratable acidity, ammonium and net acid excretion were determined according to the method of Jørgensen in an automatic Radiometer Titrator TTT 2 (11). Osmolality was estimated in an Advanced Osmometer 3D. Sodium and potassium were read on a IL 143 photometer.

RESULTS

The patient follow up over a period of seven years is shown in Fig. 3. Acid base status and calcium levels were kept within normal range; the need of oral bicarbonate progressively lessened. At present at the age of 7;10/12 years, weight and height percentiles are 97 and 90, respectively. Nephrocalcinosis is not increased. Renal function tests performed are shown in Fig. 4.

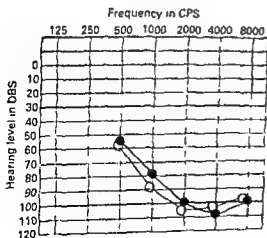


Fig. 2 Patient's audiogram

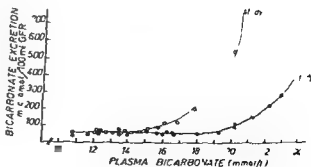


Fig. 4 Excretion of filtered bicarbonate during continuous bicarbonate infusion. The time of titration studies is de

- The syndrome of renal tubular acidosis with nerve deafness *Acta Paediatr Scand* 65 100 1976
- 7 Edelmann C M Boichis H Rodriguez Soriano J & Stark H The renal response of children to acute ammonium chloride acidosis *Pediatr Res* 1 457 1967
 - 8 Edelmann C M Rodriguez Soriano J Boichis H Gruskin A III & Acosta M I Renal bicarbonate reabsorption and hydrogen ion excretion in normal infants *J Clin Invest* 46 1309 1967
 - 9 Fiske C H & Subbarow Y The colorimetric determination of phosphorus *J Biol Chem* 66 375 1955
 - 10 Hastings A III & Sendroy J Jr The effect of variation in ionic strength on the apparent first and second dissociation constants of carbonic acid *J Biol Chem* 65 445 1955
 - 11 Jorgensen K Titrimetric determination of the net excretion of acid base in urine *J Clin Lab Invest* 9 87 1957
 - 12 Konigsmark H W Renal tubular acidosis with progressive nerve deafness Personal communication cited by McKusick V A in *Mendelian inheritance in man* Johns Hopkins Press 1971 3rd ed p 506
 - 13 Nance W E & Sweeney A Evidence for autosomal recessive inheritance of the syndrome of renal tubular acidosis with deafness *Birth Defects Original Articles* 7 70 1971
 - 14 Nash M A Torrado A D Greiser I Spitzer A & Edelmann C M Tubular acidosis in infants and children *J Pediatr* 80 738 1971
 - 15 Oetliker O & Rossi E The influence of extracellular fluid volume on the renal bicarbonate threshold *Pediatr Res* 3 140 1969
 - 16 Rodriguez Soriano J Vallo A & Garcia Fuentes M Distal renal tubular acidosis in infancy A bicarbonate wasting state *J Pediatr* 86 524 1975
 - 17 Royer P & Broyer M L acidose renal au cours des tubulopathies congenitales In J Hamburger J Crosnier & J L Funck Breton (eds) *Actualites nephrologiques de l'Hopital Necker* Flammarion Paris 1967 p 73
 - 18 Saphira E Ben Yoseph Y Eyal F G & Russell A Enzymatically inactive red cell carbonic anhydrase II in a family with renal tubular acidosis *J Clin Invest* 53 59 1974
 - 19 Sebastian A McSherry E & Morris R C Metabolic acidosis with special reference to the renal acidosis In B M Brenner & F C Rector (eds) *The kidney* W B Saunders Philadelphia 1976 p 615
 - 20 Walker W G Ozer F L & Whelton A Syndrome of perceptible deafness and renal tubular acidosis *Birth Defects Orig Artic Ser* 163 1974

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Table 3 Data from maximal free water clearance and minimal urinosmolality during hypotonic saline diuresis

	Patient age (years)	C_i (ml/min/1.73 m ²)	RTP (%)	ml/100 ml GFR					
				V	U_{osm}	C_{osm}	C_N	C_H	$C_H + C$
Study 1	5 10/12	137	84	21.8	50	4	4.2	17.8	22
Study 2	7 7/12	178	88	15.1	76	4.1	3.1	11	14.1
Normal values*									
Mean		123		14.5	65	3.4	2.5	11.1	13.6
(S.D.)		(14)		(2.1)	(12)	(0.8)	(0.7)	(1.5)	(1.9)

* C_i and RTP values prior to hypotonic saline diuresis

* Normal values in a control group of 9 children

DISCUSSION

Reports of RTA nerve deafness syndrome always refer the acidification defect as a type 1 RTA. In this patient when the first study of bicarbonate reabsorption was performed (study 1) a mixed RTA or type 1/2 hybrid was observed (19). When the study was repeated two years later (study 2) the proximal component had disappeared and the tubular defect had become an RTA type 1. Neither the abrupt increase of bicarbonate excretion observed in study 1 nor the patient's age suggest a distal RTA with bicarbonate wasting or type 3 RTA described in infants (16). The normal handling of phosphate (RTP/Tm) excludes hyperparathyroidism which could reduce proximal bicarbonate reabsorption. Two complementary facts in the patient follow up support the transformation of a mixed RTA (proximal and distal) to a classic distal RTA: 1) During the hypotonic saline expansion the excessive sodium delivery to Henle's loop (study 1) and its later normalization (study 2). 2) The progressive reduction in alkaline therapy needed to maintain normal acid base status (Fig. 3).

According to experiences of Siphira (18) a transient lack of intraluminal carbonic anhydrase activity could be considered the cause of the proximal component showed in this patient.

Early diagnosis and treatment in this patient have allowed normal growth. Hearing loss

diagnosed when he was 4 years old was not modified. This has caused a major delay in speech and social development necessitating attendance in a rehabilitation center. Late diagnosis of the hearing defect could be the cause of dumbness presented in three of the cases referred to by Dechaux (5). Donckerwolcke (6) draws attention to the necessity of revising hearing in all patients affected by distal or type 1 RTA.

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REFERENCES

1. Bijvoet O. L. M. & van der Sluis Veer J. The interpretation of laboratory tests in bone disease. *Clin Endocrinol Metabol* 1: 217, 1972.
2. Chaimovitz C, Levi J, Better O. B., Oslander L. & Benderli A. Studies on the site of renal salt loss in a patient with Bartter's syndrome. *Pediatr Res* 7: 89, 1973.
3. Cohen T, Brand Aurabin A, Karshai C, Jacob A, Gay I, Tsitsianov J, Saphiro T, Jatzvi S & Ashkenazi A. Familial infantile renal tubular acidosis and congenital nerve deafness: an autosomal recessive syndrome. *Clin Genet* 4: 275, 1973.
4. Davidson W. D. & Sackner M. A. Simplification of the anthron method for the determination of inulin in clearance studies. *J Lab Clin Med* 67: 351, 1963.
5. Dechaux M. Acidosis tubulaires primitives. In P. Royer (ed.) *Séminaire de Néphrologie Pédiatrique* Paris 1977 (abstract) p. 45.
6. Donckerwolcke M. A., Van Biervliet J. P., Koorevaar G.

NEW BOOKS RECEIVED

- J F Burke & G Y Hilditch Smith (eds) *The infectious neonatal patient* 5 pp Little Brown and Company Boston 1978 \$16.50 ISBN 0-316-11680-7
- Neil Gordon *Pediatrics: new developments in clinical medicine* Clinical Developmental Medicine No. 59/60 Spastics International Medical Publications 80 pp illus W L J. Heinemann Medical Books Ltd London 1976
- W Plöner & J Hermann *Anamnese im Kindesalter* Reihe moderne Pädiatrie 144 pp illus VEB Georg Thieme Leipzig 1977 DM 35.—
- D M Reed & F J Stanley *The epidemiology of prenatals* 370 pp illus Urban & Schwarzenberg München 1977 \$18.50 ISBN 3-541-71611-8
- J M Tanner *Foetus 1117 1117* 50 pp illus Open Books Publishing Ltd London 1978 £4.50 ISBN 0-7-91-0084-7
- P B Beeson & D A Bass *The eosinophil* Holt Saunders Ltd Eastbourne 1978 £10.50 ISBN 0-716-1650-X
- P L Morrell (ed) *Drug disposition during development* 490 pp John Wiley & Sons Ltd Sussex 1978 £14.50/\$50.00 ISBN 0-89135-006-0
- W E Bell & W F McCormick *Increased intracranial pressure in children* 2nd ed In Major Problems in Clinical Pediatrics vol VIII 472 pp illus W B Saunders Company Philadelphia London Toronto 1978 £11.00 hardback ISBN 0-7116-1708-5
- J V Simone (Guest editor) *Acute leukemia: In Clinics in Haematology* June 1978 60 pp illus tabl W B Saunders Company Ltd £16.50
- M King F King & M Martodipoero *Primary childhood cancer* A manual for health workers 315 pp illus Oxford University Press Oxford 1978 £ 00 ISBN 0-19-6479-4
- Clark A Stewart & Ann Gath *Psychological disorders of children* A handbook for primary care physicians 167 pp Williams & Wilkins Company Baltimore 1978 £10.95 ISBN 0-683-97179-9

tioned. Michael Medici and Richard Gatta describe the normal host defence mechanisms as well as defects in neonates in an easily comprehensible and highly informative way. Some of the data they have collected are difficult to find in other books. The reference list comprises 141 items. Vincent Fulginiti reviews infections in children with immune defects. For the paediatrician the last mentioned two sections are the most valuable in the book.

Some papers include original investigations e.g. chapter 9 on host defences in protein calorie malnutrition by Robert Suskind and co-workers and chapter 10 written by Gerald Bodey and co-workers describing prevention of infection in patients with acute leukemia.

The last part of the book (pp. 157-4) describing infec-

tions in surgical patients is of minor interest for the paediatrician. Changes in host defences in the immunosuppressed patient after kidney transplantation and prevention of deep wound infection following total hip replacement are reported as well as infections in surgical cancer patients.

Readers seeking basic information on immune mechanisms in infectious diseases will find this in a more comprehensible way in ordinary textbooks of immunology. But for those physicians who diagnose and treat infections in the compromised host the book is valuable as an account of the state of knowledge in this field. Many of the data collected here are indeed difficult to find in other books.

Anita Hallberg

J. M. Tanner: *Foetus into man*. Open Books Publishing Ltd. London 1978. 250 pp. illus. paperback £4.50. ISBN 0-91-0084-1.

This book deals with the fascinating and complex process of human growth from conception to maturity. The well known author has in an admirable way succeeded to describe the different aspects and problems of growth in such a way that the book can be read by people without biological knowledge and still be a highly enjoyable reading for biologically trained professionals. It is written in a clear and elegant, often amusing style, well illustrated

and with a bibliography consisting of lists of further reading and of references. The book contains twelve chapters beginning with the curve of growth and ending with disorders of growth. Sex differentiation, puberty, developmental age, endocrine control of growth etc. are presented in different chapters. For paediatricians this book is of great interest as a very comprehensive and easily read summary of what we know about growth to-day but it can also be highly recommended to all interested in human biology.

C. G. Bergstrand

A. Moragas, A. Ballabriga & M. T. Vidal: *Atlas of neonatal histopathology*. W. B. Saunders Co. Philadelphia, London, Toronto 1977. 30 pp. illus. £41.75. ISBN 0-716-634-2.

This translation of a Spanish edition from 1974 is an excellent atlas of neonatal pathology. It is of great value for both paediatricians and general pathologists. It is essentially an unusually well illustrated handbook composed of illustrative cases of mostly fatal neonatal conditions during the first month of life. Brief clinical summaries accompany detailed histopathological descriptions referring to 2-4 illustrations in full colour on adjacent plates. The photomicrographs are so well selected that the accompanying text is sufficient for comprehension without errors.

The book is based on an impressive series of 3000 neonatal cases collected in a 7 year period in Barcelona. The classical lesions of the newborn baby are illustrated both clinically and morphologically.

The book is of course devoted to the respiratory system (194 pp.) including IRDS, vascular lesions, various infections and types of asphyxia. The liver (33 pp.), kidney (3 pp.) and heart (6 pp.) are fully treated, while

rather small sections are concerned with the CNS, the GI tract and the genitalia. However, some space is devoted to all other organs.

Consequently, this will be a standard reference book in the study of neonatal pathology, mainly autopsy wise. Surgical specimens are occasionally mentioned, e.g. in a brief section on congenital tumours and on the liver.

All books have their omissions. This one has left out the largest of the baby's organs, i.e. the placenta. I would have liked it to be as well treated as all other organ systems. Only a few references are mentioned in the text and are not accompanied by a bibliography.

It was really a find to get the coup de maître—Dapena for the translation. Together they master both Spanish and paediatric pathology. Also, Mane has extra experience in the field since she published a neonatal histology atlas (in 1957 in black and white). Their pleasure in undertaking the task is evident on every page. The publishers are to be congratulated.

The high price of this atlas must be weighed against its high quality and persisting value. It treats classical lesions that we all have to deal with on all continents for many years to come.

Björn Erikmark

BOOK REVIEW

Neil Gordon *Paediatric neurology for the clinician* Clinics in Developmental Medicine No. 59/60 Spastics International Medical Publications William Heinemann Medical Books Ltd London 1976 260 pp illus

Neil Gordon has addressed his book to 'the paediatrician who is not going to practice paediatric neurology himself'. His rationale for writing the book is the estimation that a third of all paediatric consultations involve hazards to the nervous system. The author's aim has been to cover all aspects of paediatric neurology with the exception of those specific for the neonatal period.

The overall impression of this book is that it reflects

the author's vast clinical experience, also the author is very willing to present his personal opinion. The organization of the very rich material is at a first glance slightly confusing but turns out to be rather practical. The wealth of details varies between chapters again obviously as a result of the author's varying personal interest.

Paediatric neurology although it does not always provide any ready made discussions on differential diagnoses or details on pharmaceutical treatment can be recommended to any paediatrician because of its broad minded presentation, good illustrations and adequate lists of references.

Birgitta Jalling

J de Grouchy & C Tuttle *Clinical atlas of human chromosomes* John Wiley & Son New York 1977 319 pp illus £24.70 ISBN 0-471-01704-3

The rapid development in cytogenetics since the introduction of the various chromosomal banding techniques had led in recent years to an explosive and to the general paediatrician bewildering advance in the knowledge of chromosomal pathology. Much of the new information is scattered in the literature and not always easy to find. Now two distinguished cytogeneticists have brought it to gether in an admirable monograph.

The Atlas has 23 chapters and 6 appendices. One chapter is devoted to each chromosome of the human karyotype from chromosome No. 1 to a short summary, a description with illustrations of the morphology and banding pattern obtained by different techniques, the gene map, the genes assigned to this chromosome and information on the evolution of the chromosome in man and the other hominoid primates, i.e. our closest cousins the chimpanzee, the gorilla and the orang-utan. The most important part of each chapter however centers on the pathology presenting under separate headings the clinical features of old and new syndromes. Each syndrome is illustrated by photographs of patients, totally 464 photographs. References including titles appear at the end of

every syndrome presentation. The appendices include a description of current cytogenetic techniques, the basic concepts of palmar and digital dermatoglyphics terms used in clinical descriptions, chromosomal nomenclature, a list in alphabetical order of the genes located on the different chromosomes and a syndrome finder which classifies the most important symptoms and the syndromes they suggest.

The material is well presented and easy to read. The chromosomes and the syndromes for which they are responsible are described in a clear and concise way. The illustrations are with few exceptions excellent. In general the important bibliographies have been included and the references are as complete as can be expected up to 1976. My only criticism concerns Appendix III which is intended to list terms used in clinical description. The presentation here is short and shallow. The reader is however well rewarded by the wealth of information contained in the rest of the book.

This book is highly recommended for paediatrician, clinical cytogeneticists and other physicians interested in the relationship between chromosomal aberrations and congenital malformations. It is the reviewer's wish that this Atlas will be kept up-to-date every 2 years.

Felix Mitelman

John F. Burke and Gavin Y. Hildick-Smith (eds.) *The infection prone hospital patient* Little Brown and Company Boston 1978 252 pp \$16.50 ISBN 0 316 11680-7

The book consists of sixteen papers presented at a symposium devoted to the infection prone patient.

The first chapter written by Paul Que describes the role of polymorphonuclear neutrophils. Then follows a paper by Zanvil Cohn who gives a conspectus of the role of macrophages in infectious processes. Sir Gustav Nossal gives a short introduction to the cellular organization

of the immune system and has chosen to concentrate on B cell function. Data on the role of antibody in resistance to infection with *Pseudomonas aeruginosa* are given by Starkey Davis together with a count of *Pseudomonas* vaccines.

Arthur Gottlieb gives a review of transfer factor in paper 5. Chapter 6 by Wesley Alexander and co-workers has the title 'Detection of defects in host defence mechanisms and their repair in the infection prone patient'. It is a bit surprising that tests for T cell defects are described without the sheep erythrocyte rosetting test being men-

PROGENY OF SURVIVORS OF ACUTE LYMPHOCYTIC LEUKEMIA

P J MOE M LETHINEN R WEGELIUS S FRIMAN
A KREUGER and A BERG*From the Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Aurora Hospital Helsinki Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden*

ABSTRACT Moe P J Lethinen M Wegelius R Friman S Kreuger A and Berg A (Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden) Progeny of survivors of acute lymphocytic leukemia *Acta Paediatr Scand* 68: 301 1979.—Eight successful pregnancies and one spontaneous abortion have been observed in 4 women belonging to a group of 212 Nordic children who had their antileukemic therapy discontinued before January 1 1978. Further more a young leukemic man was the father of a healthy child after 4 years of intensive cytostatic therapy. No malformations have been observed in the progeny of these treated individuals.

KEY WORDS Acute lymphocytic leukemia cessation successful pregnancies offsprings

There have been dramatic advances in the treatment of acute leukemia in childhood during the past decade. Following prolonged remission it has been possible to discontinue therapy in a large number of cases of acute lymphocytic leukemia in the Nordic countries (6). Subsequently only about 20% of 212 cases have relapsed after cessation of therapy (unpublished data). The long disease free period in many of these patients and other published cases justify the contention that cure is now possible in acute lymphocytic leukemia in childhood.

Since prolonged remission or probably cure occurs in most treated cases of acute lymphocytic leukemia attention has been turned to the quality of their survival. One of the important questions in that regard is whether the reproductive function of those patients is normal. There are a few isolated reports of children born to survivors of acute lymphocytic leukemia and the incidence of defects in the offspring is not known (1, 2, 7, 8). The purpose of this presentation is to report on 8

successful pregnancies in 5 women belonging to the Nordic group of 212 leukemic children who had therapy discontinued prior to January 1978 and a young leukemic man who became the father of a healthy child after 4 years of intensive cytostatic therapy including 2 years of central nervous system therapy.

A survey is given of the material in Table 1.

CASE REPORTS

Case 1

This female was born on March 10 1947. Acute lymphocytic leukemia (ALL) was diagnosed in March 1959 when she was 17 years old. Induction therapy consisted of prednisone 90–100 mg daily and methotrexate 7.5 mg daily and maintenance therapy with 6-mercaptopurin (6-MP) 75–75 mg daily. She first became pregnant in 1965. She discontinued the 6-MP medication after about one month of pregnancy three months later she told us about her pregnancy and it was decided to let her continue the medication. The pregnancy was uneventful. Her baby a healthy girl weighing 2760 g was born on December 17 1965. 6-MP was discontinued in December 1965 after almost 7 years of antileukemic therapy. Her second child a boy weighing 2850 g was born in April 1968 and her third child a girl weighing 3100 g was

ANNOUNCEMENTS

XVI INTERNATIONAL CONGRESS OF PEDIATRICS

The XVI International Congress of Pediatrics will be held in Madrid from the 8th-13th (inclusive) of September 1980. Information can be obtained from the following address: Apartado de Correos No. 29036, Barcelona, Spain.

The 7th European Congress of Perinatal Medicine will be held in Madrid from the 14th-17th (inclusive) of September 1980. Information can be obtained from the following address: Apartado de Correos No. 79015, Barcelona, Spain.

INTERNATIONAL PRIZE FOR MODERN NUTRITION

The Central Association of Swiss milk producers announces the competition for the international prize in modern nutrition, which it is donating for 1980. The prize amounts to Sfr. 15 000 — and will be awarded to a scientist from a member state of the international dairy farming association (Austria, Belgium, Brazil, FRG, Denmark, Finland, France, Great Britain, India, Ireland, Israel, Italy, Japan, Canada, Kenya, Luxembourg, Malta, New Zealand, The Netherlands, Norway, Austria, Poland, Sweden, Switzerland, Spain, South Africa, Czechoslovakia, USSR).

The topic for 1980 is *Nutrition and Brain Development*. Deadline: February 15th 1980. Authors of articles dealing with the topic in question are invited to submit the following documents in triplicate: curriculum vitae biographic, offprints of the most relevant articles relating to the prize topic which he/she has had published in the past 5 years. The documents should be written either in German, French or English and submitted to the President of the jury, address: Prof. Dr. H. Aebi, Medizinisch-chemisches Institut der Universität Bern, CH-3000 Bern 9, Switzerland.

INTERNATIONAL SYMPOSIUM ON INFANT NUTRITION

An international symposium on infant nutrition and the development of the gastrointestinal tract will be held June 3-8 1979 at Niagara Hilton, Niagara Falls, New York.

Inquiries: Continuing Medical Education of Children's Hospital, 219 Bryant Street, Buffalo, New York 14222, USA.

EUROPEAN SOCIETY FOR SOCIAL PEDIATRICS

The Second International Congress of Social Pediatrics will be held in cooperation with the Turkish International Children's Center in Ankara June 7-9 1979. The main theme will be: Childhood accidents—epidemiology and

prevention. Deadline for registration is April 1st. Inquiries: Prof. Munneer Bertan, Hacettepe University, Ankara, Turkey.

PROGENY OF SURVIVORS OF ACUTE LYMPHOCYTIC LEUKEMIA

P I MOE M LETHINEN R WEGELIUS S FRIMAN
A KREUGER and A BERG*From the Department of Paediatrics University of Tromsø Norway Section of Hematology Tampere Central Hospital Finland Department of Paediatrics Aurora Hospital Helsinki Finland Department of Paediatrics Lapin Hospital Rovaniemi Finland Department of Paediatrics University of Uppsala Sweden Department of Paediatrics Central Hospital Karlstad Sweden*

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There have been dramatic advances in the treatment of acute leukemia in childhood during the past decade. Following prolonged remission it has been possible to discontinue therapy in a large number of cases of acute lymphocytic leukemia in the Nordic countries. Subsequently only about 20% of 212 cases have relapsed after cessation of therapy (unpublished data). The long disease free period in many of these patients and other published cases justify the contention that cure is now possible in acute lymphocytic leukemia in childhood.

Since prolonged remission or probably cure occurs in most treated cases of acute lymphocytic leukemia attention has been turned to the quality of their survival. One of the important questions in that regard is whether the reproductive function of those patients is normal. There are a few isolated reports of children born to survivors of acute lymphocytic leukemia and the incidence of defects in the offspring is not known (1, 2, 7, 8). The purpose of this presentation is to report on 8

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Table 1 *Survey of the material*

Case no	Sex	No of children	Abortion	Age at diagnosis (y)	Duration of anti-leukemic therapy (y)
1	F	3		12	6½
2	F	1	1	12	10
3	F	2		7	10
4	F	1		18	4½
5	F	1		15	5½
6	M	1 ^a		21	6½ (until death)
Total		9	1		

^a The mother on therapy at the time of the first conception

^b The father on therapy at conception

born in September 1973. All her 3 children are normally developed and in good health. This case has been reported previously (8).

Case 2

A female was born on February 2, 1955. ALL was diagnosed January 19, 1968. Induction therapy consisted of prednisone 60 mg daily and maintenance therapy of 6-MP 125 mg daily until April 1969 when she relapsed. Treatment with a combination of vincristine 2 mg weekly and prednisone 60 mg daily was started and a complete remission was achieved again. Maintenance treatment with 45 mg methotrexate twice weekly was initiated in April 1969. Methotrexate was discontinued in September 1970 due to pulmonary infiltrations and 6-MP therapy 125–50 mg was reinstituted. Antileukemic therapy was stopped in January 1976 after 10 years of cytostatic therapy. In March 1977 the patient gave birth to a healthy girl weighing 4380 g and both the mother and child have later been completely well.

Case 3

A female was born on April 13, 1956. ALL was diagnosed in October 1963. Induction therapy consisted of prednisone 80 mg daily and 6-MP 75 mg daily and maintenance therapy of 6-MP 50–100 mg daily. After 10 years of cytostatic therapy treatment was discontinued in October 1973. Her first child, a boy weighing 2910 g, was born in 1974 about one year after cessation of therapy and her second child, a boy weighing 3070 g, was born in 1976. Both children are healthy and normally developed.

Case 4

A female was born on October 6, 1967. ALL was diagnosed in January 1972. Induction therapy consisted of prednisone 60 mg daily, 6-MP 75 mg daily and vincristine 2 mg once weekly and maintenance therapy of 6-MP 75 mg daily. The maintenance treatment was decreased to 75 mg five times weekly on March 1972. One year later cranio-spinal irradiation with 2200 rad was given. Antileukemic therapy was discontinued in September 1976. She became pregnant in January 1977 and gave birth to a healthy boy weighing 3460 g in October 1977. The mother and child have been in good health since.

Case 5

A female was born on September 3, 1953. ALL was diagnosed in December 1968. Induction therapy was initiated with prednisone 90 mg daily and 6-MP 100 mg daily. Maintenance therapy consisted of 6-MP 100 mg daily. All therapy was discontinued in March 1974. She had a spontaneous abortion in second month in February 1976. In August 1977 she gave birth to a boy weighing 3190 g. He had slight calcaneo-valgus deviation of both feet. Both the mother and the son are otherwise well.

Case 6

A male born on October 1, 1948. ALL was diagnosed in August 1970. Induction therapy with prednisone 80 mg daily and vincristine 4 mg once weekly was initiated and he received maintenance therapy with 6-MP 140 mg–100 mg daily. Central nervous leukemia was diagnosed in January 1973 and he received intensive therapy with methotrexate intrathecally. He was also in 1973 given 3 courses of COAP (cytosine arabinoside 140 mg daily, prednisone 200 mg daily, cyclophosphamide 200 mg daily for 5 days and vincristine 2 mg once). Conception took place while he was on therapy. He married and had a two months prematurely born daughter in December 1975 weighing 2100 g. Blood group studies indicated that the patient was the father of the child. The patient was continued on therapy until he died in March 1977 from hematologic relapse 6½ years after therapy was initiated. His daughter has developed normally and is currently healthy.

DISCUSSION

Now when cure is possible in children with ALL, attention must be focused on the late effect of antileukemic therapy. An important question to these young patients is what prospects of reproduction they have and the possible effects of different types of antileukemic therapy upon the development of the fetus.

The children in long term remission from acute leukemia seem to have a good chance of normal development of puberty particularly those diagnosed in early childhood (7). There are however reported only a few cases of offspring of survivors of acute childhood leukemia (1, 2, 7, 8). We have now reported 9 offsprings (now 1-13 years old) of 6 survivors of ALL, all of whom are normal. Only one of the ten pregnancies ended with spontaneous abortion. We do not know however whether abortion has occurred in any of the other survivors of ALL. A study reported by Li & Jaffe on the progeny of 45 childhood cancer survivors showed no excess of children with defects (5). That study included however only two cases with leukemia.

The five females received a relatively conservative type of treatment regimen with moderate intensive induction and only one drug as maintenance. None of our cases receiving high doses of methotrexate have so far become pregnant. Case 4 received cranio-spinal irradiation. Our male patient had received intensive cytostatic therapy including high dose of methotrexate and three courses of COAP (including cyclophosphamide) before the conception. It is usual in the Nordic countries today to discontinue antileukemic therapy after 3 years of continuous remission. Most cases of leukemia in childhood occur before the age of ten years. Children above 13 years are in fact high risk cases with a small chance of long term survival. The problem of offsprings of such cases off therapy will therefore be rare even in the future. We must however calculate on numerous pregnancies in children who have terminated their anti-leukemic therapy here in the Nordic countries in recent years (6).

It seems important to collect data on all pregnancies in long term leukemia survivors and try to obtain information on their offsprings. This study is too limited in number to permit any sweeping conclusions, but the initial results are reassuring. More studies are required before any definite conclusion can be

drawn both as to long term prognosis in leukemia survivors and the possible effects of the more intensive cytostatic treatment regimens on pregnancy.

It is too early to ascertain whether central nervous system irradiation or the use of high dose methotrexate will cause fetal damage.

More data are needed concerning the long term effects of the disease and the effects of different types of cytostatic therapy both on the patients and their offsprings. There are some data which indicate that close to 20% of children receiving irradiation for malignancy will develop a second malignancy within 20 years (5). It is however too early to calculate the risk of secondary malignancy in children receiving intensive cytostatic therapy for acute leukemia. Finally nothing is known as to whether offsprings of leukemic survivors have increased risk of neoplasia. Answers to these vexing questions will be apparent only after these treated patients and their children have been under surveillance for many years.

REFERENCES

- 1 Bacon C & Kernahan J. Successful pregnancy in acute leukemia. *Lancet* II 515 1975.
- 2 Editorial. The price of survival in childhood leukemia. *Br Med J* I 371 1978.
- 3 Li F P. Second malignant tumors after cancer in childhood. *Cancer* 40 1899 1977.
- 4 Li F, Cassidy J R & Jaffe N. Risk of second tumors in survivors of childhood cancer. *Cancer* 35 1 30 1975.
- 5 Li F F & Jaffe N. Progeny of childhood cancer survivors. *Lancet* II 707 1974.
- 6 Moe P J. Cessation of therapy in childhood leukemia. A survey of a Nordic material consisting of 16 cases. *Acta Paediatr Scand* 67 145 1978.
- 7 Sims E S, Leventhal M G & Vaitukaitis J L. Effects of childhood leukemia and chemotherapy on puberty and reproductive function in girls. *N Engl J Med* 94 1143 1976.
- 8 Wegelius R. Successful pregnancy in acute leukemia. *Lancet* II 1301 1975.

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QUALITY OF CARE A TRACER DIAGNOSIS STUDY OF ACUTE OTITIS MEDIA COMPARING A DISTRICT PAEDIATRIC SERVICE WITH PAEDIATRIC AND OTOLARYNGOLOGY EMERGENCY DEPARTMENTS

■ OLIN P BOLME G EWERT B LAGERKVIST ■ STERKY
K TENGVOLD and R ZETTERSTRÖM

From the Department of Paediatrics St Goran's Hospital the Graduate School of Social Work and Public Administration and the ENT Department Sabbatsberg's Hospital Stockholm Sweden

ABSTRACT Olin P Bolme G Ewert B Lagerkvist B Sterky ■ and Zetterstrom R (Department of Paediatrics St Goran's Hospital Stockholm Sweden) Quality of care A tracer diagnosis study of acute otitis media comparing a district paediatric service with paediatric and otolaryngology emergency departments *Acta Paediatr Scand* 68 305 1979 Acute otitis media was used as a tracer condition for comparing patient care ■ regards patient satisfaction compliance and medical outcome in a district paediatric office an otolaryngological and a paediatric hospital emergency department The initial work up of the 216 patients studied at the three units was in good agreement with predefined standards However while the district paediatrician arranged a follow up for all patients the hospitals did so only in 10-30% of the visits Parent satisfaction was significantly higher at the district office when the time spent waiting and the time the patient was with the doctor were kept constant Urinary penicillin was not detected in 1/17 of district paediatric and in 6/19 of otological patients Compliance with follow up was also much higher at the district paediatrician Medical outcome did not differ between the units The majority of the parents expressed preference for the type of care ■ en ■ a neighbourhood service It is suggested that the criteria for adequate treatment of acute otitis media adapted for emergency departments may be revised in case easy access is provided to a medical centre offering continuity of care and proper follow-up Studies of the quality of care have so far not managed to demonstrate a definite and positive relationship between the process of care patient satisfaction and final medical results

KEY WORDS Quality of care otitis media district paediatric service

The concept of quality of care is poorly defined and the variables that are included in such studies differ considerably among different authors (7 9 22 23 26) The process of medical care may be evaluated by measuring medical outcome and patient satisfaction Brook & Appel (6) related the long term outcome for specific diagnoses to the type of care given as compared with established criteria for diagnosis and treatment They showed that the outcome was poorly related to adherence to established medical norms Becker et al (2) showed that continuity of care in a hospital setting was associated with higher parent satisfaction and better compliance In primary care where patients often follow courses of treatment prescribed by the

medical team positive patient or parent satisfaction may be necessary to achieve compliance and adequate medical outcome Patient satisfaction may also be considered as a goal in its own right since medical services are expected to satisfy the requirements of patients These are some reasons why patient satisfaction and for paediatric practice parent satisfaction is regarded as an important measure of the quality of care (2 14)

The aim of this study is to compare the quality of care in a district paediatric service with hospital facilities This report is part of an evaluation of a district paediatric service with integrated health and sick care for a defined child population in a suburb of Stockholm (13)

Table 1 Summary of issues studied and type of data used

Issues	Data
Structure of medical service i.e. training, working hours, waiting time etc.	Questionnaire to physicians
Content of medical care Diagnostic procedures compared to professional standard	Questionnaire to physicians
Measures and recommended follow up	Questionnaire to physicians Questionnaire to parents
Parental perception of care Satisfaction	Questionnaire to parents Interview with parents 5 months later
Family compliance with Medication Follow up	Urinary penicillin determination Chart review Contact with the physicians Interview with parents 5 months later
Medical outcome Number of visits within 5 months Hearing test	Chart review Play audiogram
Social background	Questionnaire to parents

We used a tricer diagnosis design (12). Acute otitis media was selected as the tricer condition since approximately 20 to 25% of first visits to the district paediatric ward and 6% to the paediatric emergency department were for treatment of this condition (13). In addition this diagnosis is well defined. The beneficial effect of adequate treatment was considered well established and final outcome easily determined (12).

We collected information on the following factors (Table 1): the structure and content

of medical services, the parents' satisfaction and compliance with the regime and the final medical outcome, in order to study how the organization of medical services influences the quality of care given. Background data on patient characteristics were also obtained in order to assess selective demands for medical services.

MATERIAL

Medical services studied

The comprehensive district paediatric service was situated in a newly built suburb about 12 km from the

Table 2 Patients studied at three clinics

Medical unit	Patients studied	Parent question naire	Telephone interview	Urinary penicillin	Audio gram ^b
District paediatric	56	48	46	17	15
Paediatric emergency department	93	77	77	—	11
Otolaryngological emergency department	67	60	59	19	41
Total	216	185 (86%)	182 (84%)	36	67

Urinary penicillin determined in children older than 3½ years within an accessible geographic area. The three children at the paediatric hospital who were eligible were not included in the analysis. Nine children at the district paediatrician's office and five at the otological hospital were not reached in time for the urine sample. Two otolaryngological patients refused.

^b Pure tone audiograms were performed on children older than 3½ years. Eleven were not reached and two refused to participate.

Table 7 Demographic and social data of the families

Variable	District paediatrician		Paediatric emergency department		Otolaryngology emergency department	
	N	%	N	%	N	%
Age of child	46		93		67	
<1 years		16		30		8
1-3 years		43		40		30
>3 years		41		70		63
Marital status of mother	46		73		59	
Married or cohabitant		93		88		100
Single		7		12		17
Age of mother	45		74		58	
<20 years		0		4		3
20-29 years		69		64		52
>29 years		31		37		45
Educational level of mother (school years)	48		75		58	
>12		6		12		10
10-12		56		44		35
<10		38		43		55
Working hours of mother	47		71		59	
Fulltime		23		23		44
Part time (1-35 hrs/week)		55		39		31
Not employed		21		38		25
Social class (father)	43		67		46	
I		19		18		22
II		23		2		13
III		54		54		59
Not classified		4		6		6
Tenure	46		73		59	
Rental		59		61		64
Owner occupied		41		39		36
Bedroom standard	46		73		59	
≤1 person/room		54		58		66
1-2 persons/room		46		41		33
>2 persons/room		0		1		0

centre of Stockholm. The office was staffed by a paediatrician (Board certified 1971) and a paediatric registered nurse and was open four hours per day. Mondays through Fridays. The paediatrician was working in the adjacent child health centre and was the school physician for the same population during the remaining office hours on weekdays. All families were living within walking distance of the paediatric service. After office hours emergency care was available at the two hospitals studied.

The otolaryngological emergency department is located in a general hospital in the centre of Stockholm. The paediatric emergency unit belongs to a university department located in another hospital. Both are open 24 hours a day. Emergency care is primarily given by several physicians in postgraduate training. Physicians and auxiliary staff work in the emergency room on a rotating schedule. It is therefore impossible to provide continuity of care in most instances.

General sickness insurance covers the visits to all clinics studied. The fee was 15 Sw. kr per visit.

In the end of September 1974 data collection was started in the office of the district paediatrician for two weeks, followed by one month of simultaneous recording at all three services and concluded by three weeks of collection at the district paediatric service. The extended time compensated for the smaller catchment area of the neighbourhood service.

Patients studied

Table 7 shows the number of patients participating in the study as described below under Methods.

According to the background data obtained from the questionnaires answered by parents, some differences in family characteristics were found between the units (Table 3). The paediatric emergency department was more frequently visited by children less than one year old. The mothers were often not working outside the home. The otolaryngological department saw more often older children with older working mothers. The families visiting the district paediatrician were intermediate in

Table 4 *Criteria for diagnosis of acute otitis media*

The diagnoses were used in the following order: purulent, simplex, non-specific otitis, otosialpingitis when physical findings within several categories were noted.

	Otitis			Otosialpingitis
	Purulent	Simplex	Non-specific	
<i>History</i>				
Ear discharge	x			
Acute earache, no positive physical finding			x	
<i>Physical findings</i>				
Ear discharge	x			
Drum abnormalities				
Perforation	x			
Bulging	x			
Red		x		
Dull and thickened		x		
Red malleus		x		
Retracted				x
Clear fluid in the middle ear				x
Decreased movement				x
Unspecified abnormalities			x	
Spore trigus			x	

several variables. Thus the district paediatrician served as an alternative to both emergency departments. In the following analysis we will only compare the district paediatrician with the two hospital units and refrain from matching the latter. The socioeconomic differences did not seem to influence our main findings. They show, however, that the type of clinic used is selected to some extent to suit the mother's working conditions.

METHODS

We agree upon criteria for the diagnosis: treatment and follow-up of acute otitis media before the data collection started. This was in accordance with Swedish and international studies (3, 3, 19, 24) and local otolaryngology praxis. Acute otitis media according to our criteria may be divided into purulent otitis, simplex otitis and non-specific otitis (Table 4). The table also includes the criteria for otosialpingitis.

The initial care was considered adequate when at least ten days of treatment with antibiotics in combination with nasal and/or oral decongestive agents were prescribed and a follow-up visit was arranged. In case of otosialpingitis, decongestive therapy and arrangements for follow-up sufficed (3).

Data concerning diagnosis and initial treatment were collected with questionnaires to the participating physicians at the visit. Participating physicians were informed

about the aim of the study and asked to complete a questionnaire for each child aged six months to 15 years with acute otitis media or otosialpingitis according to his/her own assessment (13). No more than three symptoms and three signs supporting the diagnosis were to be included in the questionnaire. In addition to the diagnostic tests used, drugs presented and follow-up arrangements made. Data on the duration of the visit and the patient's general condition were also collected.

The parents were given written information about the study at the time of the visit and were asked to participate and to answer a questionnaire mailed to their homes within five days (13). Background factors about the patient, parent preference and satisfaction were derived from this questionnaire and from a telephone interview with the parents five months later.

Compliance was checked by chart reviews regarding follow-up visits and in some instances by determination of penicillin in the urine during treatment. Urine samples for control of treatment were obtained by a unannounced telephone call from children over two and one half years of age and living in the suburbs close to the district paediatrician. The samples were collected at home within one hour, frozen and later analyzed for penicillin by a microbiological technique (9, 11).

At the telephone interview five months after the visit the parents were questioned about the child's current condition and hearing. The response rate for the parent questionnaire and interview was 82-90% at the different units (Table 2). A major reason for non-participation was language difficulties. The study was not aimed at the specific problems of ethnic minorities. To elucidate such questions other methods should be used. The remaining nonparticipants included the abstaining parents and comprised less than 10% of the material.

The number of medical contact made because of ear trouble since the index visit and complications such as chronic otitis, serous otitis and remaining perforation or an otological operation or adenoidectomy were recorded by chart reviews after six months. Children older than 5 years were offered an audiometric examination five to six months after the visit.

After coding, cross-tabulation and statistical calculation were performed employing an IBM 60 programme (18). χ^2 values corresponding to $p < 0.05$ were considered statistically significant and $p < 0.001$ were highly statistically significant.

RESULTS

Medical process

Most children had a simplex otitis according to our criteria (Tables 4 and 5). The proportion of children with purulent otitis was of the same magnitude in the district paediatrician's office and the otolaryngological emergency room. The general condition of the children was rated fair to good by the physicians at all

Table 5 Patients with otitis at different clinics subgrouped according to criteria in Table 1

Medical unit	Otitis			Otosal pyngitis (%)	Not class- ifiable (%)	Total	
	Purulent (%)	Simplex (%)	Nonspecific (%)			(n)	N
District paediatrician	27	64	7	0	2	100	56
Paediatric emergency department	17	75	5	0	2	99	93
Otolaryngological emergency department	8	63	5	3	2	101	67
Total	33	68	6	1	2	100	216

clinics There was no evidence that more serious illnesses were selectively seen by otologists Most patients (90% of the 210 with otitis) were prescribed antibiotics for ten days Of the 24 children who were not given antibiotics none had purulent otitis according to our criteria Treatment was similar at all three units

The district paediatrician arranged follow up visits for all of her patient at her office

The otolaryngological emergency department arranged follow up visits at the hospital for 30% and at the paediatric emergency department for less than 10% Seventy percent of the patients at both hospitals were advised to contact another physician

Parent preferences

In the questionnaire the parents were asked to select between pairs of mutually exclusive alternatives of medical service By this

technique different aspects of the neighbourhood and hospital services were juxtaposed such as continuity of care versus proximity to consulting experts easy access to daytime service versus 24 hour service and proximity to home versus hospital resources About half of the families wanted a neighbourhood medical service with the same doctor supplemented by a 24 hour emergency facility However 50% of those who had experience of the district paediatrician thought it possible to wait 24 to 48 hours before consulting a doctor in the case of acute otitis—a condition which according to many otologists requires emergency treatment (Table 6)

Parent satisfaction

How did the expressed preference for the neighbourhood centre correspond to the parents attitudes towards the service given at the different units? The parents were

Table 6 Parental preferences for the provision of care for acute otitis according to questionnaire

n = number of parents responding to all relevant questions

Unit	Neighbourhood service (%)	Neighbourhood service + emergency room (%)	Hospital + continuity of care (%)	Hospital (%)	Other (%)	Unclassified (%)	Total (n)	n
District paediatrician	50	40	0	0	0	10	100	40
Paediatric emergency department	5	47	8	4	8	11	100	57
Otolaryngology emergency department	9	57	15	2	11	6	100	44
Total	19	48	16	2	7	8	100	141

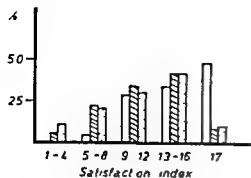


Fig. 1 Parental satisfaction with the visit to the doctor and auxiliary staff according to questionnaire and interview. The index was based on ten questions regarding the parent's opinion of the visit in general and of the physician and nurse(s) performance with respect to contact with the child, helpfulness, information given and time spent. A high score indicates high satisfaction. Percentages for each unit. Open bars = district paediatric visits ($N=40$), hatched bars = paediatric emergency visits ($N=59$), stippled bars = otolaryngological emergency visits ($N=49$).

much more often satisfied with the visit to the district paediatrician than the visits to hospitals. This was also true of their appreciation of the doctor's management of the patient and the auxiliary staff's activity. The answers to several questions regarding parental satisfaction were compiled into indexes for the three units (Figs 1 and 2). The higher satisfaction expressed for the service given by the district paediatrician could be due to various structural differences. The waiting time there seldom exceeded one hour (5%) and was usually less than 20 min (65%). In the emergency departments a waiting time of one hour or more was common (34–54%), the longest waiting time being in the department of paediatrics. Parents who had to wait more than one hour were less satisfied. However, variations in waiting time within one hour did not affect satisfaction at each unit.

The district paediatrician spent slightly more time with her patients. The doctors at the otolaryngological emergency department were quickest. However, there was no relation between the short time spent by the doctor and requests for more time or information. The differences between the district paediatrician and the hospitals regarding satis-

fication were still statistically significant when waiting time was below one hour and doctor's time was 6–15 min. This shows that other factors are involved in parental satisfaction.

Patient compliance

Determinations of penicillin in the urine on the fifth to the eighth day of treatment were performed in 19 children from the otolaryngological department and in 17 from the district paediatrician. Six of the hospital children as compared to one of the district paediatrician's children had no measurable quantity of penicillin in the urine. Thus 1/3 of the hospital children did not have adequate treatment as compared with 1/17 at the neighbourhood service. The difference is significant at the 5% level using Fisher's exact test. The difference could be related to the confidence in the district paediatrician's office as suggested by the analysis of parent satisfaction. This was also illustrated by the district paediatric nurse's own explanation: 'When a mother phones and tells me that she cannot give penicillin to her child, I just tell her to come down and I'll show her how to do it.'

Follow up of otitis is often considered to be a necessary part of treatment in order to

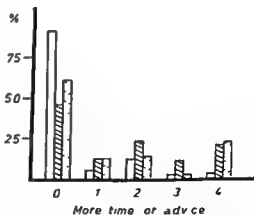


Fig. 2 Parental requests for more time or advice at the visit according to the questionnaire. The figures correspond to the number of requests made for more time or advice from either the physician or the nurse. Percentages as in Fig. 1. District paediatric visits ($N=47$), paediatric emergency visits ($N=45$), otolaryngological emergency visits ($N=47$).

reduce the risk of future loss of hearing. The chart review showed that 98% of the district paediatrician's children had a follow up as compared to only 54% of the otolaryngological and 42% of the paediatric hospital patients. The differences between the district paediatrician and the hospitals were highly significant. Parent satisfaction within each clinic was not related to whether follow up was performed or not.

Medical outcome

Eleven of the 93 paediatric emergency patients were referred to an otolaryngological department or underwent an otological operation or adenoidectomy within six months. Corresponding figures for the district paediatric patients were nine of 55 and for the otolaryngological emergency patients 14 of 62. The differences between the neighbourhood and the hospital patients were not statistically significant. However, among patients attending a scheduled follow up visit, the proportion of later complications was highest among the otolaryngological patients. Twelve of 26 underwent an operation as compared to nine of 55 district paediatric ($p < 0.01$) and six of 34 paediatric emergency patients. Thus the paediatric patients received the same amount of specialist care irrespective of follow up. The otolaryngological emergency department on the other hand had a larger proportion of patients with complications among those who made follow up visits. The result may indicate that the otologists more accurately selected the patients requiring follow up. In addition, the families may themselves compensate for inadequate follow up at the paediatric hospital.

After five to six months, two of 15 children at the district paediatric service showed a hearing loss of at least 25 decibels in either ear as compared with four of 11 paediatric hospital patients and five of 41 at the otolaryngological emergency unit. The differences were not statistically significant. The patient's satisfaction was not related to the

medical result as measured by residual hearing loss or otological complications.

GENERAL DISCUSSION

Families that attend emergency departments at hospitals prefer to some extent the type of care given by the district paediatrician. The preference is more marked in persons who have visited the neighbourhood centre. This result is in accordance with Skinner et al. (20) and emphasizes the importance of direct referrals from the hospitals to the relevant neighbourhood centre.

The diagnostic and therapeutic programme at the district paediatrician's office was adequate for the diagnosis studied and surpassed the hospitals by offering an adequate follow up.

The neighbourhood service met many of the demands expressed by the parents. Waiting time was shorter and more time was spent by the doctor. Continuity of care was provided during office hours, since the same physician and nurse examined the patients, whereas in hospitals the same doctor seldom saw the patient at the infrequent follow up visits.

The diagnostic precision and the initial treatment varied less than expected at the different clinics. In a study of paediatric hospital care of gastroenteritis, great differences between reasons for admission and duration of hospital stay were found (21). Similarly, in studies initiated in response to the legislation of professional standards review organizations in the USA, emphasis has been placed on showing the great variations in existing medical care rather than in demonstrating the deviation from a given professional norm (25). The conformity may mirror the uniform teaching in the departments of otolaryngology at medical schools and the efficiency of the postgraduate programmes in Sweden.

However, the criteria used for treatment of otitis media should be questioned. Treatment

with antibiotics for ten days and the compulsory follow up visit may not be necessary in all cases of uncomplicated otitis provided easy access to a neighbourhood medical centre can be made available in case of complications. Rigid adherence to specified medical norms adjusted to a system offering fragmented and non continuous medical care may prove unnecessary and costly where an efficient neighbourhood service is provided.

This study demonstrates the deficiencies in care given at the emergency departments studied especially the disregard for the effect of discontinuity of care and unsatisfactory follow up routines on patient compliance. The results are in agreement with several studies from emergency departments on children treated with penicillin for acute tonsillitis or otitis where 30–50% did not complete prescribed treatments according to various criteria (2, 4, 8, 10). Earlier studies were focused on patient factors. Any hypotheses that the variations in satisfaction and compliance reflect variations in social background knowledge about infections and level of expectation among the families at the three clinics are not confirmed by our data. Rather the results are in accordance with the supposition that the local paediatric service was in a better position to carry out a regime than the hospital emergency unit.

More extensive studies of the quality of care although imperfect (15, 16, 17) are important means for clarifying weaknesses in the existing medical system and for suggesting alternative programmes for treatment in improved settings.

REFERENCES

1. Armstrong M W. The treatment of acute middle ear infection in children. *Otolaryngol Clin North Am* 3: 249, 1970.
2. Becker M H, Drachman R H & Kirscht J P. Predicting mother's compliance with pediatric medical regimens. *J Pediatr* 81: 843, 1972.
3. Bergholtz L. Allmänna principer för utbehandling.
4. In R D, Rudberg & H Hallander (eds). *Symposium över diagnostik och behandling av akut otit mitt och tonsillit*. Astra Lakemedel, Södertälje, 1973, p. 141.
5. Bergman A B & Werner R J. Failure of children to receive penicillin by mouth. *N Engl J Med* 68: 1334, 1963.
6. Bolme P & Eriksson M. Influence of diarrhea on the oral absorption of penicillin V and ampicillin in children. *Scand J Infect Dis* 7: 141, 1975.
7. Brook R H & Appel F A. Quality of care assessment: choosing a method for peer review. *N Engl J Med* 78: 1323, 1973.
8. Donabedian A. Evaluating the quality of medical care. *Milbank Mem Fund Q* 44: no. 2, 166, 1966.
9. Elling R, Whitmore R & Green M. Patient participation in a pediatric program. *J Health Hum Behav* 1: 183, 1960.
10. De Geyndt W. Five approaches for assessing the quality of care. *Hospital Administration* Winter 1970: 21, 1970.
11. Gordis L, Markowitz M, Lichtenfeld A M. The inaccuracy in using interviews to estimate patient reliability in taking medications at home. *Med Care* 1: no. 2, 49, 1969.
12. Jalling B, Malmberg A S, Lindman A & Bureus L O. Evaluation of micromethod for determination of antibiotic concentrations in plasma. *Eur J Clin Pharmacol* 4: 150, 1972.
13. Kessner D M & Kalk C E. *Contrasts in health status: vol. 2. A strategy for evaluating health services*. Institute of Medicine, National Academy of Sciences, Washington D C, 1973, p. 11.
14. Lagerkvist B, Olin P, Tengvald A, Sterky G & Zetterstrom R. *Distriktsbarnläkare. Studier av vård utnyttjande och vardskvalitet i en storstadsförort*. Sprit rapport 11, 1976.
15. Lebow J L. Consumer assessments of the quality of medical care. *Med Care* 12: 328, 1974.
16. Lindsay M I Jr, Hermans P B, Nobrega F T & Ilstrup D M. Quality of care assessment: I. Outpatient management of acute bacterial cystitis as a model. *Mayo Clin Proc* 51: 307, 1976.
17. McNemey W J. The quandary of quality assessment. *N Engl J Med* 295: 1505, 1976.
18. Morehead M A. Ambulatory care review. A neglected priority. *Bull N Y Acad Med* 52: 60, 1976.
19. Nie N H, Hull C H, Jenkins J G, Steinbrenner K & Bent D H. *Statistical package for the social sciences*. SPSS, New York, 1975, 2nd ed.
20. Rudberg H D. Acute otitis media. Comparative therapeutic results of sulphonamide and penicillin administered in various forms. *Acta Otolaryngol* Suppl. 113: 1, 1954.
21. Skinner T J, Price B S, Scott D W & Gorry G A. Factors affecting the choice of hospital based ambulatory care by the urban poor. *Am J Public Health* 67: 439, 1977.
22. Smedby H & Sundelin C. Admission rate and length of stay for nonspecific enteritis in pediatric departments in Sweden. *Int J Health Serv* 7: 537, 1972.

- Starfield B & Scheff ■ Effectiveness of pediatric care: The relationship between processes and outcome *Pediatrics* 49:547 1972
- 3 Thorner R M Health program evaluation in relation to health planning *Health Serv Rep* 86:525 1971
- 24 US Dept Health Education and Welfare Hearing sensitivity and related medical findings among children *Vital Health Stat II* no 14 1972
- 25 Welch C E, Arena J M, Locco A, Light F V, Lucas F, Porterfield J D, Shalowitz M, Slee V N & Steinholtz L H PSRO and norms of care *JAMA* 229:166 1974
- 6 WHO *Methods of evaluating public health programmes* Report on a symposium Kiel November 1967 WHO Europ 0375 1968

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REFERENCES

1. Armstrong H W. The treatment of acute middle ear infection in children. *Otolaryngol Clin North Am* 3: 249 1970.
2. Becker M H, Drachman R H & Kirscht J P. Predicting mother's compliance with pediatric medical regimens. *J Pediatr* 81: 843 1972.
3. Bergholtz L. Allmänna principer för utbehandling.
4. In R D, Rudberg & H Hallander (eds). *Symposium over diagnostik och behandling av akut otitissmitt och tonsillit*. Astra Lakemedel, Södertälje 1973 p 141.
5. Bergman A B & Werner R J. Failure of children to receive penicillin by mouth. *N Engl J Med* 288: 1734 1963.
6. Holme P & Eriksson M. Influence of diarrhea on the oral absorption of penicillin V and ampicillin in children. *Scand J Infect Dis* 7: 141 1975.
7. Brook R H & Appel F A. Quality of care assessment: choosing a method for peer review. *N Engl J Med* 288: 1323 1973.
8. Donabedian A. Evaluating the quality of medical care. *Milbank Mem Fund Q* 44: no 3: 166 1966.
9. Elling R, Whitmore R & Green M. Patient participation in a pediatric program. *J Health Hum Behav* 1: 183 1960.
10. Geyndt W. Five approaches for assessing the quality of care. *Hospital Administration* Winter 1970: 21 1970.
11. Gordis I, Markowitz M, Lilienfeld A M. The inaccuracy in using interviews to estimate patient reliability in taking medications at home. *Med Care* 7: no 2: 49 1969.
12. Jalling B, Malmberg A S, Lindman A, C Boreus L O. Evaluation of micromethod for determination of antibiotic concentrations in plasma. *Eur J Clin Pharmacol* 4: 150 1972.
13. Kessner D M & Kalk C E. *Contrasts in health status: vol 2. A strategy for evaluating health services*. Institute of Medicine, National Academy of Sciences, Washington D C 1973 p 11.
14. Lagerkvist H, Olin P, Tengvald K, Sterky H & Zetterstrom R. *Distriktsbarnläkare. Studier av vård utövande och vårdkvalitet i en storstadstjänst*. *Sprirapport* 11 1976.
15. Lebow J L. Consumer assessments of the quality of medical care. *Med Care* 12: 378 1974.
16. Lindsay M I, Jr, Hermans P E, Nobrega F T & Ilstrup D M. Quality of care assessment. I. Outpatient management of acute bacterial cystitis as a model. *Mayo Clin Proc* 51: 307 1976.
17. McNeerney W J. The quandary of quality assessment. *N Engl J Med* 295: 1505 1976.
18. Morehead M A. Ambulatory care review. A neglected priority. *Bull NY Acad Med* 52: 60 1976.
19. Nie N H, Hull C H, Jenkins J G, Steinbrenner K & Bent D H. *Statistical package for the social sciences*. SPSS, New York 1975 2nd ed.
20. Rudberg R D. Acute otitis media. Comparative therapeutic results of sulphonamide and penicillin administered in various forms. *Acta Otolaryngol Suppl* 113: 1 1964.
21. Skinner T J, Price H S, Scott D W & Gorry G A. Factors affecting the choice of hospital based ambulatory care by the urban poor. *Am J Public Health* 67: 439 1977.
22. Smedby B & Sundelin C. Admission rate and length of stay for nonspecific enteritis in pediatric departments in Sweden. *Int J Health Serv* 2: 537 1972.

- 9 Starfield H & Scheff D : Effectiveness of pediatric care: The relationship between processes and outcome *Pediatrics* 49 547 1972
- 10 Thorner E M : Health program evaluation in relation to health planning *Health Serv Rep* 86 575 1971
- 11 US Dept Health Education and Welfare : Hearing sensitivity and related medical findings among children *Vital Health Stat II* no 14 1972
- 12 Welch C III, Arena J III, Cocco A, Light F V, Lucas F, Porterfield J D, Shalowitz M, Slee V N & Steinholtz L H : PSRO and norms of care *JAMA* 229 166 1974
- 13 WHO : *Methods of evaluating public health programmes*. Report on a symposium Kiel November 196 WHO Europ 0375 1968

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THE PROGNOSIS OF NEAR DROWNED CHILDREN

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ABSTRACT Kruius S Bergstrom L Suutarinen T and Hyvonen H (Research Department Rinnekoti Institution for the Mentally Retarded Espoo Department of Neurology University of Helsinki and Children's Hospital University of Helsinki Finland) The prognosis of near-drowned children *Acta Paediatr Scand* 68 315 1979.—Thirty children were treated for near-drowning in the Children's Hospital University of Helsinki during 1971–1976. The patients were divided into 3 groups according to the prognosis: group I included 13 children (43%) with a favourable prognosis; group II four children (13%) with a less favourable prognosis who developed severe sequelae; and group III 13 children with poor prognosis and in whom the subsequent outcome proved fatal. The surviving children underwent neurological, neurophysiological and psychological examination 6–48 months after the accident. The children in group I had slight neurological or psychological signs; some children presented a lowered intellectual functioning level. The children in group II were tetraplegic, unable to speak and had convulsions. The following factors were important in affecting prognosis: the longer the immersion time, the worse the prognosis. However, prognosis could still be favourable with an immersion time of 11–20 min. Prognosis was bad if the first pH value was <7.00. The arterial oxygen pressure values measured during the treatment did not correlate with the prognosis but a low rectal temperature on admission was usually associated with a bad prognosis. The degree of EEG-disturbance had a prognostic value. However, the follow-up recordings correlated better with the prognosis than the recordings during the first 24 hours, after which worsening of the EEG sometimes showed a progressive brain lesion.

KEY WORDS Near-drowning children

Drowning in Finland as in many other countries is one of the three leading causes of death in childhood. Every year a number of near-drowning victims are treated in children's hospitals. The prognosis for a complete recovery is held to be very good (4–13). In the material of 36 near-drowned children Enksson *et al.* found two seriously disabled cases. In a series of 29 near-drowned patients of all ages Modell (13) confirmed that there were no neurological deficits. In the former study the sequelae were estimated by interviewing the parents; in the latter study the main interest was concentrated on following pulmonary function after near-drowning. The aim of our study is to present the clinical findings in the acute period after the near-drowning and to consider the

prognosis of near-drowned children using neurological, neurophysiological and psychological examinations.

During the progress of this work Modell *et al.* (14), Fandel & Bancalari (5) and Pearn (16) published their material concerning the prognosis for near-drowning.

MATERIAL AND METHODS

The material consists of 30 children who were admitted to the Children's Hospital University of Helsinki because of near-drowning during 1971–1976. The age distribution was 7 months–13 years 11 months; median 4 years 4 months. Twenty-three (77%) of them were boys; seven (23%) girls (Table 1).

All the children had been submerged in water and consciousness had been lost. Twenty patients were brought to the Children's Hospital directly following first aid; the

Table 1 Distribution of near drowned children according to age and sex

Age (years)	Sex		Total
	Boys	Girls	
<1	2		2
1-4	10	4	14
5-9	7	3	10
10-14	4		4
Total	23	7	30

other ten were transferred from other hospitals within 20 hours of the accident.

Eight of the children had been found in a lake, seven in a ditch or pit, seven in a swimming pool, four in the sea, three in a waterbucket and one in a river.

The Baltic seawater near the Finnish coast contains a very small amount of sodium chloride (0.02 to 0.75‰). The submersions in the sea happened near Helsinki. Thus, it could be said that all the accidents in this series belong to the category of freshwater drownings.

The information concerning the clinical course in the acute period was collected from hospital records. All 17 children who survived were re-examined. The period from the accident to re-investigation varied from 6-68 months (median 22 months, mean 29 months). The re-examination included an interview with the parents and an EEG recording, as well as the clinical neurological examination and psychological testing.

Psychological tests were performed on 13 children. WISC (Wechsler Intelligence Scale for Children) was applied to 9 children. TML (the Version of Termán Merni tests standardised in Finland) was chosen in three cases and WPPSI (Wechsler Preschool and Primary Scale for Intelligence) in one case. To measure the visual motor coordination, Bender was used in 11 cases and drawing simple geometric figures was used in the two youngest patients.

The intelligence of the four most handicapped children was estimated by a child neurologist.

An estimation of pre-accident intellectual capacity was made from the interview with the parents. In one case pre-accident psychological testing was available.

A total of 91 EEG recordings were performed on 28 patients. Twenty patients were examined within 24 hours of the accident. Following this, 24 patients were examined between 24 hours to 2 weeks after the accident, and 20 patients thereafter. At the time of the first EEG recording some patients were being artificially respiration.

The EEG patterns were classified according to Pamphogne (15). Class 1 includes the recordings in which the activity mainly corresponded to that of age limits or showed only slight slowing. Class 2 includes the recordings in which the brain activity was mainly in the delta range. Class 3 includes the recordings in which brain activity was composed of periods of irregular slow waves with or without spikes or sharp waves. Between the periods of activity, silent periods lasting a few or several seconds were obtained. Class 4 includes isoelectric EEGs.

RESULTS

The material has been divided into three groups according to the subsequent outcome (Table 2). Group I includes children whose prognosis had been favourable (13 patients, 43%). Group II includes those patients with severe neurological sequelae (4 patients, 13%). Group III consists of those children who died in hospital (13 patients, 43%).

Correlation of the immersion time to the outcome (Table 3). The exact immersion time was known only in one case. In 18 cases it could be estimated but in 11 cases estimation was impossible. The approximate immersion time was 5 minutes in seven cases, all of which belonged to group I. In one case hypoxia lasted 15 min longer than the immersion time because of a delay in beginning resuscitation.

Four children were immersed for between 10 to 10 min—in three of them the prognosis had been favourable while one had severe sequelae.

Table 2 Appearance of neurological and psychological signs in near drowned children

P, pre-accident signs; +, slight; ++, marked; +++, severe; —, no abnormal findings.

Group	Pat.	Neurological findings	Psychological findings	
			Lowering of general IQ	Failure in visuomotor coordination
I	2	P	—	P
	7	+	+	++
	8	+	—	++
	9	+	—	—
	14	+	+	++
	16	P	—	P
	20	+	+	++
	21	—	—	+
	22	—	—	P
	24	—	—	+
II	28	—	—	++
	29	—	—	P
	30	P	—	P*
	1	P, +, ++	+++	+++
	13	+++	+++	+++
	19	+++	+++	+++
	27	+++	+++	+++

* Reading and writing disorder.
* Spatial difficulties.

Table 3 Immersion time according to prognosis in near drowned children

Immersion time (min)	Favourable prognosis (Group I)	Severe sequelae (Group II)	Fatal outcome (Group III)	Total
≤5	7			7
6-10	3	1		4
11-20	3	1	4	8
No estimation		2	9	11
Total	13	4	13	30

In one case a delay in resuscitation of about 15 min

lae Eight children were immersed for 11 to 20 min. Of these three had a favourable prognosis although there were some signs of a brain lesion. One patient had severe sequelae and four died.

Of the 11 cases in which the immersion time could not be estimated all had a bad prognosis, two ending with severe sequelae and nine with death.

Clinical findings in the acute period (Table

4). In group I two patients did not need any resuscitation, seven were resuscitated immediately after being brought out of the water or on the way to hospital, and in four cases resuscitation had to be continued in hospital. In one case where the rectal temperature of the patient was 25°C the heart started to beat but arrested later and the patient had to be re-resuscitated.

Six patients in group I required mechanical ventilation and four of them had PEEP (positive end expiratory pressure). The ventilation lasted from 2 to 4 days except in two cases where a pneumothorax complicated the therapy and the ventilation was thus prolonged to eight days. All patients in groups II and III needed mechanical ventilation. In half of the cases PEEP was used. In group II the duration of the ventilation varied from 4 to 9 days.

Corticosteroid treatment was used in eight patients in group I and in all the patients in groups II and III. Signs of pulmonary oedema were observed in the X-ray examination of 27

Table 4 Data concerning the patients with a favourable prognosis (group I) in the acute period and the neurological findings on re-examination after near drowning

Case	Age (years)	Immersion time (min)	Mechanical ventilation (days)	Return of consciousness	Seizure res.	Other neurological signs in the acute period	Neurological findings in the re-examination
3	10			At scene		Slight spasticity in all limbs, coordination failure, absence fits, Sluggish	Same as in the acute period
7	8	0	+	6 h			Coordination failure, sluggish
8	1	10	1+8	21 d		Tetraplegia spastica	Coordination failure
9	5	5	3	4 d	+		Coordination failure, Babinski + at right
14	3	15	1+6*	10 d		Hypotonia musculorum, Hemiparesis leviss, 1 dx nystagmus strabismus	Coordination failure, Same as in the acute period
16	13	5	4	1 d	+	Dystonia, tetraplegica	Coordination failure, athetoid movements
0	5	15	3	3 d			Normal
1	9	10		5 h	+		Normal
4	5	5		At scene, 3 h			Normal
8	8	3		At scene			Normal
20	9	3		At scene			Normal
10	8	3		At scene		Stumbling gait, headache	Normal, Coordination failure, spatial difficulties

Pre-accident signs
Pneumothorax on 1st day

* Delay in beginning resuscitation was about 15 min

† Pneumothorax on 3rd day

Table 5 Initial blood pH values according to prognosis in near drowned children

Initial blood pH values	Favourable prognosis (Group I)	Severe sequelae (Group II)	Fatal outcome (Group III)	Total
<7.00		1	6	7
7.00-7.14	12	2	1	17
Unknown	1	1	4	6
Total	13	4	13	30

patients. Three patients belonging to group I had no pulmonary oedema.

The initial blood pH values taken within 2 hours of the accident are presented in Table 5.

Repeated arterial oxygen (P_{aO_2}) measurements were made in most of the cases (Fig. 1). P_{aO_2} values did not correlate with the prognosis.

The first rectal temperature measurements on admission to the hospital are presented in Fig. 2. The lowest value was 25°C. Thirteen patients had rectal temperatures below 30°C; in all these cases the immersion time had been longer than 5 min and 11 patients were immersed during the cold season. It seems that a low initial rectal temperature is often associated with a bad prognosis.

Neurological state in the acute period (Table 4). Eight patients in group I were unconscious on admission. Three patients returned to full consciousness on the same day and three within 4 days. In two patients the period of unconsciousness was longer (10 and 21 days) and both suffered the complication of pneumothorax (cases 8 and 14). The patients in groups II and III were all unconscious on admission. None of the patients in group III returned to full consciousness before death. Many of the children had seizures during the acute period.

Six patients in group I showed no neurological signs after consciousness returned. The other seven had neurological symptoms, two of them showing signs that had existed previously. The short case reports of the five patients with neurological signs caused by immersion are as follows (Table 4). **Case 7.** A

6-year old girl was very slow in her reactions and speech. She has improved slightly. **Case 8.** An 18-month old boy had spastic tetraplegia two weeks after the accident. After six weeks he began to walk and later to speak. **Case 14.** A 3 1/2-year old boy with the complication of a pneumothorax had marked muscle hypotonia and could not speak. Four weeks later he could walk and speak, but his muscle power was still not good. **Case 20.** A 5 1/2-year old boy still had a tendency to opisthotonus, severe dystonic tetraplegia and choreoathetosis four

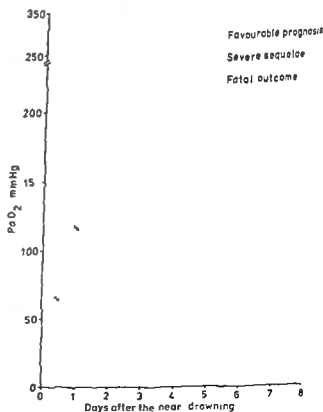


Fig. 1 Arterial oxygen tension values in near drowned children.



Fig 2 Rectal temperature on admission to the hospital and the prognosis of near drowned children

weeks after the accident. He neither spoke nor could he fix his eyes. Two months later he began to walk and speak and his vision was good. Case 30: A 8 year old boy was discharged home on the third day after the accident. His gait was stumbling and he suffered severe headache on the fifth day after the accident when corticosteroid treatment was discontinued. The headache lasted four days. The gait was normal after five days.

The neurological and psychological findings on re examination (Tables 2 and 4) On re examination five children out of 17 survivors had no neurological symptoms. Five children in group I who had had neurological findings in the acute period still had slight neurological signs (coordination failure) at the time of re examination. It appeared from the interview with the parents that three other patients in group I with neurological signs had already had the same neurological signs before the accident (cases 2, 16 and 30).

In group I all the patients underwent psychological testing. The median of the general IQ was 96 (mean 99.2, range 88 to 115, Fig 3) except for two children whose IQs were low (48 and 76, cases 2 and 16) and whose intelligence was already low before the accident. In three cases the functioning level was estimated to be lower than before the accident. These patients had marked specific difficulties and/or a lack of concentration.

In the WISC all the other patients showed

corresponding verbal and performance scores except for two in whom differences were seen. In Bender's test seven children in group I had failure of visual motor coordination. Failure was marked in five of them and slight in two.

In group II all patients were mentally retarded and tetraplegic with difficulty in swallowing. They could not move or speak. All of them reacted to auditory stimuli and recognised their mothers' voices. Three did not react to visual stimuli but none had optic atrophy. Three had seizures despite anticonvulsant medication. All except one were living in hospitals. One of these children had pre-accident psychomotor retardation but had learned to walk before the accident.

In group III ten patients died within ten days and three lived in a decerebrate state before dying of pneumonia within 8 months of the accident. All three had severe cerebral necrosis on autopsy.

EEG findings (Table 6) In group I eight patients did not show any abnormalities in the acute period or at re examination. In five patients there was a slight general slowing in the EEG. Two of them had a normal EEG recorded within 24 hours of the accident but the EEG recorded one week later showed moderate slowing. One of them (case 30) complained of headache. On re examination all patients in group I showed normal EEG activity except for one in whom paroxysmal activity was shown in the EEG 3 1/2 years later. This patient had the complication of pneumothorax during the acute period.

In group II two patients were examined

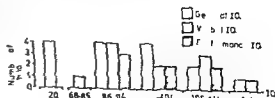


Fig 3 Intelligence levels in 15 near-drowned children. The verbal and the performance IQ was measured in nine children.

Table 5 Initial blood pH values according to prognosis in near drowned children

Initial blood pH values	Favourable prognosis (Group I)	Severe sequelae (Group II)	Fatal outcome (Group III)	Total
<7.00		1	6	7
7.00-7.34	12	2	3	17
Unknown	1	1	4	6
Total	13	4	13	30

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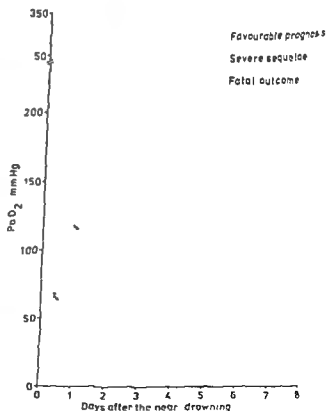


Fig. 1 Arterial oxygen tension values in near drowned children.

cellent and that 95% of the surviving children are neurologically normal

In this Finnish study the outcome was more often fatal and post accident neurological deficits were seen more often than in other materials. Our patients were treated like Modell's (14) and Fandel's (5) patients after the principles evaluated by Modell (13). Modell points out that near drowning in fresh water affects pulmonary function. Washing out of the surfactant factor leads to atelectasis and there are risks of pulmonary oedema, bronchial obstruction, pneumonitis and pneumonia. In nearly all of our cases the arterial oxygen pressure could be held within a safe area and there was no block in ventilation, pulmonary function or in the pulmonary circulation. The brain lesion was the crucial factor. In Pearn's series (16) the children were immersed in freshwater but the immersion times were much shorter than in this Finnish experience, i.e. from 0.5 to 10 min (mean 3.7 min). In our material the immersion time could not be calculated exactly except in one case. In addition, after the children are brought out of the water there may have been delay in the beginning of resuscitation or resuscitation may not have been done correctly. Both these factors lengthen the duration of an inadequate blood supply to the brain. It has been suggested that submersion for longer than five minutes is uniformly fatal (11). Results in the monkey after one hour's complete experimental ischemia provide evidence that it may be possible to survive much longer immersion (7).

Cases of survival without sequelae have been reported after submersion for 17, 20, 22 and even 40 min (8, 9, 10, 18). The cause of recovery in these cases was held to be due to hypothermia resulting from falling into cold water.

In the Finnish material hypothermia was not a good prognostic sign. Seventy percent of the patients with an initial rectal temperature of less than 30°C had a bad prognosis, as opposed to 25% of the patients with a rectal

temperature of 35°C or more. It can be concluded that hypothermia, like a low blood pH value, indicates a long immersion time and more severe brain anoxia.

There is little information regarding re-warming to be found in the available literature. From our experience active re-warming should not be done quickly and should not exceed 32°C. At this temperature the heart functions well but the brain has a reduced oxygen requirement. The seizures seen in four patients with a favourable prognosis occurred during active re-warming, indicating an increased need of the brain for oxygen (1, 3).

In this study the first EEG recordings were of some prognostic value: the EEGs of the patients who survived were normal or only slightly disturbed. The recordings from the patients who died showed more pathological brain function.

In the follow-up the EEGs of the patients who had recovered had returned to normal despite having been initially disturbed. In the patients with severe sequelae or who died the EEG had worsened. In many cases the worsening appeared in the form of paroxysms. However, the initial worsening of the EEG was not always indicative of severe neuropsychological sequelae. In two patients there had been a transient general slowing but in the follow-up period the EEG had returned to normal. The clinical symptom of the slowing period of the EEG was headache. At this time no disturbances in the lung function could be detected. Thus some other mechanism other than late lung oedema must be the cause of this phenomenon.

On the basis of our experience we think that EEG evaluation is a very useful method for assessing the prognosis of near-drowning. The follow-up recordings correlated better with the prognosis than did recordings within 24 hours of the accident, after which time a worsening of brain function could still occur. This is inconsistent with findings on the prognostic value of the EEG in brain anoxia after cardiocirculatory arrest, where the rate of

Table 5 EEG changes (1-4 see text) in various prognostic groups correlated with the time interval after near drowning

Prognostic groups	EEG classes Time after near drowning		
	24 hours	1-14 days	>14 days
Group I			
Favourable prognosis	1	1	1
	1	1	1
	1	1	1
		1	1
		1	1
	1	2	1
	1	2	1
	2	2	1
	2	2	1
		2	1
Group II	1	2	1
		2	1
Severe sequelae	1	2	2
		2	3
Group III			
Fatal outcome	1	1	4
	1		
	2	2	1
	2	4	
	2	4	
	3	4	
	3	4	
	4	4	
	4	4	

With paroxysms

within 24 hours of the accident and both of them showed normal brain activity. However, within two weeks a worsening of condition had occurred leading to a class 2 EEG. Two other patients were examined for the first time during the week following the accident. Both of them were classified as class 2. On re-examination more than one month after the accident the EEGs of all the patients in group II were pathological. Three had paroxysmal activity and one showed a flat EEG.

In group III 11 patients were examined within 24 hours of the accident. Six of them had an EEG pattern which was classified as class 3 and 4 and the other five patients were classified as 1 and 2. In all patients the EEG became progressively worse or showed no re-

covery except in one patient who died four months later.

DISCUSSION

Two factors affecting accident proneness in childhood are raised by this study. The first is one of sex. Boys were victims in 77% of the cases, same tendency being found in other childhood accidents (12-19). Biological factors and upbringing make boys more active than girls. Even small boys are expected to be independent and to play alone while girls are more restricted by being held closer to the mother (6).

The other factor affecting accident proneness would appear to be neuropsychological. According to the parents, six of the surviving 17 children had some preaccident neuropsychological sign. Three children had subnormal intelligence with neurological signs. Two of them had difficulty in reading and writing and one had spatial difficulties. Failure in perception and motor coordination make such children unable to cope with dangerous situations.

According to this study the prognosis after near drowning is favourable when the immersion time does not exceed 10 min, the initial blood pH value is above 7.00 and the patient regains consciousness fairly rapidly (11) within a few days. In the acute period they may show marked neurological symptoms which resolve. Their EEG recordings are normal or slightly altered.

In this study 56% of the patients survived. The prognosis was favourable in 43% and 13% of the patients had severe neurological sequelae. The outcome was fatal in 43% of the cases. In Modell's series (14) of 91 near drowning patients 89% survived and two patients had neurological deficits. In Frindel's and Brunel's material (5) 82% of the children survived, 12% had severe neurological deficits, findings which are the same as in our material. Pearn (16) maintained that the prognosis of near drowning in childhood is ex-

CLINICAL COURSE OF WHOOPING COUGH IN CHILDREN YOUNGER THAN SIX MONTHS

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ABSTRACT Trollfors B (Department of Infectious Diseases Östra Sjukhuset Göteborg Sweden) Clinical course of whooping cough in children younger than six months. *Acta Paediatr Scand* 1979; 68: 323-328. The patient records of 59 children aged 2-26 weeks with culture verified pertussis were analysed. Twenty four of them were hospitalized in most cases for social reasons. Only one child with hypothyroidism and a complicating pneumonia was critically ill. Seventeen of the 35 non hospitalized patients had a mild disease without developing typical whooping attacks. Thirteen children were treated with erythromycin in the catarrhal stage. There was a tendency towards milder disease in this group but the differences compared to untreated children were not statistically significant.

KEY WORDS Pertussis. Infants.

Despite a high immunization rate whooping cough is still a common disease in many countries. In Sweden there has been an impression among clinicians that the disease has become milder during the last two decades even though it has become more common. This is also indicated by a study by Strangert (5). From the United Kingdom Miller & Fletcher (6) have reported that pertussis is still a serious disease in children under six months but has a benign course in older patients. As children aged under six months seem to have more severe disease this study was undertaken to evaluate the severity of the disease in this age group.

PATIENTS AND METHODS

At the Department of Clinical Bacteriology in Gothenburg Sweden which serves an area of about 700 000 inhabitants *B. pertussis* was isolated in nasopharyngeal cultures from 1 630 patients between January 1 and December 31 1977. Sixty four of them were children aged below six months. Patient records were available for study in 49 of these cases (29 boys 31 girls). The children had been seen in the University Hospital in Gothenburg two smaller hospitals (Mölndal and Uddevalla) and nine pediatric outpatient clinics. When the patient records were incomplete additional information was obtained from the parents.

RESULTS

Hospitalization The frequency and reasons for hospitalization in different age groups are summarized in Table 1. The 24 hospitalized children had a mean period of hospital stay of 7.5 days (range 1-24 days). If four patients with longer stay for other reasons (hypothyroidism serious social problems) are excluded the mean duration of hospital stay was somewhat less than five days. The child with the complicating pneumonia (8 weeks¹) was the most critically ill. He was hospitalized one week after the beginning of the whooping attacks with extensive pulmonary infiltrates. For the first 24 hours he was kept in an oxygen tent. After two days he was already markedly improved but stayed in hospital for four weeks as he had a congenital hypothyroidism and a suspected cardiac lesion requiring further investigation.

Two of the children admitted because of apnoea and cyanosis improved quickly and were discharged after two and five days respectively. The third child in this group had a

Age when symptoms started

EEG improvement in the first few hours after the resuscitation seems to have the best prognostic value (15).

In patients with cardiac arrest the duration of brain ischaemia has been shorter than in those with near drowning, in whom many factors before the cardiac arrest (such as respiratory changes in water electrolyte balance, changes in blood pressure) can affect oxygen supply to the brain. The time interval before effective resuscitation must also have been longer in near drowning.

In connection with brain ischaemia, increasing intracranial pressure caused by brain swelling, aggregation of blood particles and morphological changes can decrease the circulation (7) even with good lung function. It is possible that both the transient and the progressive slowing in the EEGs obtained in our patients can be explained on this basis.

Thus, the brain lesions in near drowning can be compared to those of brain contusion where a worsening can happen 3–4 days after the accident corresponding to the development of brain oedema.

Finally, recent reports on the treatment of cerebral ischaemia with high doses of barbiturates are most interesting. In this treatment when the circulation is restored, cerebral function is immediately depressed by giving large doses of thiobarbiturates. Primate studies indicate a very marked improvement compared to previous results, and there are also reports on the very favourable effects of this treatment on humans. This should be held in mind when planning the future treatment of near drowning victims (2, 17).

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REFERENCES

- Bigelow W G, Lindsay W K, Harrison R C, Gordon R A & Greenwood W F. Oxygen transport and utilization in dogs at low body temperatures. *Am J Physiol* 160: 125, 1950.
- Brenvik H. Feasibility of barbiturate loading of patients for brain resuscitation after cardiopulmonary arrest. Abstracts of the Second World Congress on Intensive Care, Paris 19–23 Sept. 1977, p. 165.
- Brendel W. Kreislauf in Hypothermie. *Verh Dtsch Ges Kreislaufforsch* 33: 33, 1957.
- Eriksson B, Fredin H, Gerdman P & Thorsen J. Sequelae of accidental near drowning in childhood. *Scand J Soc Med* 1: 3, 1973.
- Fandel I & Bincalari E. Near-drowning in children: clinical aspects. *Pediatrics* 58: 473, 1976.
- Goldberg S & Lewis M. Play behaviour in the very old infant: early sex differences. *Child Dev* 40: 91, 1969.
- Hossmann K A & Zimmermann V. Resuscitation of the monkey brain after 1 h complete ischaemia. I. Physiological and morphological observations. *Brain Res* 91: 59, 1974.
- Imbich P, Kahus K & Tonz O. Erfolgreich! Behandlung eines Ertrinkungsfalls nach 11 Minuten Submersion. *Schweiz Med Wochenschr* 103: 160, 1975.
- King R B & Webster I W. A case of recovery from drowning and prolonged anoxia. *Med J Aust* 1: 919, 1964.
- Kvittingen T D & Næss A. Recovery from drowning in fresh water. *Br Med J* 1: 1315, 1963.
- Leugheed D W, Jones J M & Hall G E. Physiological studies in experimental asphyxia and drowning. *Can Med Assoc J* 40: 423, 1949.
- Marcusson H. Epidemiologie des Unfalls im Kindesalter. *J Gesamte Hyg* 13: 222, 1967.
- Modell J H. *The pathophysiology and treatment of drowning and near drowning*. Charles C Thomas, Springfield, Ill, 1971.
- Modell J H, Graves S A & Ketover A. Clinical course of 91 consecutive near drowning victims. *Chest* 70: 231, 1976.
- Pimpiglione G & Harden A. Resuscitation after circulatory arrest: prognostic evaluation of early EEG findings. *Lancet* 1: 1761, 1969.
- Pearn J. Neurological and psychometric studies in children surviving freshwater immersion accidents. *Lancet* 1: 7, 1977.
- Safir P & Nemoto E. Brain resuscitation: a summary of its present status. 14th Congress of the Scandinavian Society of Anaesthesiologists, Uppsala, Sweden, June 29–July 2, 1977.
- Siebek H, Brenvik H, Rod T & Lind B. Survival after 40 minutes submersion without cerebral sequelae. *Lancet* 1: 1275, 1975.
- World Health Organization. *The prevention of accidents in childhood*. Report on a WHO seminar, Copenhagen 1960.

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Immunization Thirty one children had had one two or three injections of pertussis vaccine while 24 were not immunized. Information was lacking in four cases. The development of whooping attacks, feeding difficulties or need for hospitalization could not be related to immunization status.

Erythromycin treatment One child (six weeks') had been given erythromycin prophylactically because of pertussis in the family. Two days after having finished a ten day course he developed symptoms. He was later hospitalized for five days because of feeding difficulties. Three children were given erythromycin on the very first day of catarrhal symptoms. One of them had a very mild disease with no whooping attacks. The second child developed moderately severe whooping attacks while the third child developed typical whooping attacks with rather severe vomiting. Ten children were given erythromycin on the second to fifth day of catarrhal symptoms. Five of them had a mild disease without typical whooping attacks. Twenty nine children were given erythromycin to reduce contagiousness after more than five days of symptoms usually when the whooping attacks had already started. One of them had to be hospitalized because of diarrhoea.

Laboratory data Blood leucocytes in 26 children (20 hospitalized) in the whooping stage ranged from 10 2-31 0 (mean 22) $\times 10^9/l$ with 55.5 to 88 (mean 76) % mononuclear cells. Seven children in the catarrhal stage with symptoms for less than one week had 7.8-11.1 (mean 9.6) $\times 10^9$ leucocytes/l with 33-51 (mean 43) % mononuclear cells.

DISCUSSION

As only patients with positive cultures were included in this study the results might not give a completely true picture of pertussis in small children in this region. In mild cases with no known exposure to pertussis the diagnosis might not be suspected while on the other hand in clinically typical cases culture

might be considered unnecessary. It is not likely though that the most serious cases have been missed as they should have appeared at one of the hospitals all of which have been interested in pertussis with a high frequency of cultures.

It may therefore be concluded that pertussis in Sweden even in the youngest age group is in most cases a mild to moderately severe disease causing more social than medical complications. Of the 59 patients only one with a congenital hypothyroidism was critically ill while as many as 19 never developed typical whooping attacks. Secondary infections occurred in only five cases.

There are many possible explanations for the declining severity of the disease. Isler et al (3) and Miller & Fletcher (4) have shown that active immunization is of value but their results as well as the findings in this study show that even complete immunization does not always protect from typical whooping attacks. Whether or not immunization with the Swedish vaccine predisposes to a milder course is not possible to evaluate from this uncontrolled material in which only six children were completely immunized. Other possible reasons for the decreasing severity of the disease suggested by Isler et al (3) are antimicrobial therapy, improved nursing care and supportive therapy and perhaps a decreased virulence of the causative organism.

Antibiotics are certainly of value for the treatment of secondary bacterial complications. Erythromycin treatment in the catarrhal stage might also affect the subsequent course of the disease as shown by Bass et al (2). The children treated early in this study had a somewhat milder course as six out of 13 did not develop whooping attacks compared to 13 out of 46 children not treated in the catarrhal stage but this difference was not statistically significant. The fact that one child developed symptoms two days after having completed a ten day course of erythromycin given

Age when symptoms started

Table 1 *Reasons for hospitalization in children with pertussis in different age groups*

Reasons for hospitalization	Age group			
	2-8 weeks	9-16 weeks	17-26 weeks	Total
Social reasons	3	6	4	13
Feeding difficulties and vomiting	2	2	1	5
Apnoea	0	2	1	3
Concurrent pneumonia	1	0	0	1
Fever	0	0	1	1
Diarrhoea (erythromycin induced)	0	0	1	1
Total number hospitalized	6	10	8	24
Non hospitalized children	11	9	15	35
Total	17	19	23	59

complicating obstructive bronchitis and had to be kept for observation for three weeks.

The five children hospitalized because of feeding difficulties and serious vomiting were admitted in the second to fourth week of the disease. Three of them had lost weight. All improved satisfactorily and could be discharged after four to ten days in good condition.

None of the other 15 hospitalized children had severe disease with apnoea, secondary bacterial complications or serious feeding difficulties. One of them was admitted because of diarrhoea caused by erythromycin given to reduce contagiousness. Another was observed for two days because of fever which disappeared spontaneously. Thus 13 children were hospitalized mainly for social reasons because the parents were worried or worn out after being kept awake by the nightly coughing attacks. Most of these 13 children had moderately severe whooping attacks, sometimes with cyanosis, but were in good general condition and alert between the coughing attacks. Two of them never developed typical whooping attacks at all. One of the mothers of these children had pertussis herself and six parents had more than one child with the disease, which explains why they could not take care of their children at home. In three of these cases hospitalization lasted from two to four weeks owing to serious social problems (a pair of twins had a mother with a serious men-

tal disorder and a girl had a father hospitalized after having been assaulted).

Non hospitalized patients As many as of the 35 patients not requiring hospitalization had a mild disease with no typical whooping attacks and little or no vomiting. The reason why nasopharyngeal cultures were obtained was catarrhal symptoms or nonspecific cough in children who had been exposed to pertussis in the family. The second youngest child (three weeks¹) belonged to this group. The other 18 of the 35 non hospitalized children had typical whooping attacks, often with short periods of cyanosis and vomiting, but were in good general condition. The youngest child (two weeks¹) belonged to this group.

Secondary infections As already mentioned in the hospitalized group there was one case of bronchopneumonia and one case of obstructive bronchitis. A non hospitalized child had a small bronchopneumonia (verified by X ray) which was cured without antibiotic treatment. One child had an otitis and one had a tonsillitis (beta-haemolytic streptococci cultured).

Exposure to pertussis Thirty six of the 59 children had caught the disease from older brothers or sisters. Seven children had been infected by cousins or children of neighbour. In 16 cases the source of infection was not known.

¹ Age when symptoms started

with whooping cough. However this boy had a history of IRDS in the neonatal period.

It is always hard to judge the severity of a disease in a retrospective study. The absence of recorded complications does not necessarily mean that hospitalization was mainly for social reasons. Only rarely in the notes of our 57 infants were social reasons mentioned as a contributory reason for admission. Most infants required individual supervision by nurses able to give oxygen and use suction when necessary. We share the opinion of other investigators that the relative lack of complications should be attributed to improved nursing care and not only to the fact that whooping cough may be milder.

There seems to be general agreement that infants should be protected from whooping cough. A general recommendation has been erythromycin for the index case to reduce its contagiousness and for the infant to possibly prevent the disease. However we have noticed as has the author that in many infants (in his study 16/59 i.e. one third) the source of infection is not known. The only means of protecting these infants is to reduce the incidence of the disease in older children by immunization. This is why the Swedish Pediatric Association recommends that pertussis vaccine should still be used.

Several antibiotics have been shown to be effective against *Bordetella pertussis* in vitro. Unfortunately few clinical trials have been conducted to test its effect. The drug of choice today erythromycin has been shown to rapidly eradicate the bacteria from the throat during treatment (2, 3). However in comparison with drugs that were used earlier there are only a few patients that have been followed after discontinuation of erythromycin. I think that many doctors today would welcome such a study in order to be able to better calculate the risk for exposed individuals.

In two studies erythromycin was shown to be effective in preventing the spread of whooping cough among exposed babies in a nursery (1, 4). This lead to its widespread use

in Sweden. In accordance with the findings of the authors of the study in Gothenburg we have also seen whooping cough in prophylactically treated infants. Among 15 infants admitted to our hospital during 1978 three received this treatment at the same time as the index cases (siblings). They developed whooping cough in spite of this and had to be hospitalized for medical reasons.

Margareta Eriksson

Dr Trollfors wants to make the following comments

After having read the comments made by Dr Eriksson I would like to discuss some points. Unfortunately Dr Eriksson misquoted me in writing. The author concludes that whooping cough is a mild to moderately severe disease. The correct citation should have been: *is in most cases a mild to moderately severe disease* as I am aware that serious cases exist and also reported one in the study.

I do not think that the material described by Dr Eriksson presents a worse picture of the disease than mine. She found 56 hospitalised cases in 8 years whereas I found 24 hospitalised cases in one year even though the hospital in Stockholm received children for intensive care from a region twice as large as that of Gothenburg. The main difference between our studies is that Dr Eriksson described only hospitalised cases while I tried to cover the whole spectrum of the disease including non hospitalised cases. I feel that Dr Eriksson underestimates our colleagues on pediatric departments in writing. The absence of recorded complications does not necessarily mean that hospitalization was mainly for social reasons. Complications serious enough to motivate hospitalisation would surely have been noted in the records on admittance or discharge.

prophylactically is remarkable as Altmeier & Ayoub (1) have shown good effect of prophylactic erythromycin treatment

In the whooping stage erythromycin is only indicated to reduce contagiousness or to treat secondary bacterial complications. It has probably been prescribed too widely, as most of these children came from families in which other susceptible members already had the disease or must have been exposed before treatment started. Almost all hospitalized children were treated with erythromycin even though they were kept separate from other children. This might be justified if members of the staff are susceptible.

The good nursing care at a modern pediatric hospital is naturally of value for children with serious whooping cough. Most cases reported here, though, did not require hospitalization. Many of the other infants stayed in hospital for only a few days and left with unchanged symptoms, so the period of hospital stay could not possibly have affected the general course of the disease.

Thus even in the youngest most susceptible age group there are many whooping cough patients with mild symptoms who have not been immunized, not received early erythromycin treatment and only received basic nursing care from their parents at home. This leaves room for speculation that the benign course of pertussis seen during the last two decades is due to non-medical factors such as a generally improved resistance to infections related to better socio-economic conditions and/or a decreased virulence of the causative organism.

REFERENCES

- Altmeier W A & Ayoub E M. Erythromycin prophylaxis for pertussis. *Pediatrics* 59: 623, 1977.
- Bass J W, Hlenk E L, Kotheimer J B, Linne M, Cronin C C & Smith M H III. Antimicrobial treatment of pertussis. *J Pediatr* 75: 768, 1969.
- Isler J, Anglin C E & Middleton P J. The whooping cough syndrome: A continuing pediatric problem. *Clin Pediatr* 14: 171, 1975.
- Linnemann C C, Ramundo N, Perlstein P H, Minton B D, Englander G S, McCormick J B &

- Hayes P S. Use of pertussis vaccine in an epidemic involving hospital staff. *Lancet* II: 540, 1975.
- Melchior J C & Jensen O. Hospitalsindryk af 14 hosteepidemier 1973-1974. *Ugeskr Læger* 138: 185, 1976.
- Miller C L & Fletcher W II. Severity of notified whooping cough. *Br Med J* 1: 117, 1976.
- Sirnail A P, Penier L & Grege R H. The use of parenteral feeding in whooping cough. *Clin Pediatr* 17: 409, 1978.

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The Editor has asked Dr Margareta Enksson, Karolinska Institutet, St. Goran's Children's Hospital, Stockholm, Sweden, to comment on the article on whooping cough.

Whooping cough has achieved new and increasing interest during the last few years. In most countries in the Western world it is still considered to be a serious disease in infants below the age of three to six months (3, 5, 6, 7). The author of this retrospective survey concludes that whooping cough in Sweden is a mild to moderately severe disease. As long as there is no generally accepted definition of what is a serious disease, opinions will differ between investigators as well as with the group of infants studied.

During the past eight years 57 infants less than six months of age (28 below 3 months) have been admitted to St. Goran's Children's Hospital. This hospital serves a population of 400,000 and in addition receives children for intensive care from an area with a population of 1 1/2 million. Of these 57 infants three developed such serious apneic spells that artificial ventilation was required for a period of 4-21 days. All of the infants were full-term healthy babies of six to seven weeks of age. In addition, during this period artificial ventilation was also required for a 10-month-old boy.

A PROSPECTIVE STUDY ON THE INCIDENCE AND SIGNIFICANCE OF CONGENITAL CYTOMEGALOVIRUS INFECTION

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M PEDERSEN³ O ØSTERBALLE⁴ U FELSAGER and E MOGENSEN

From the Institute of Medical Microbiology ²Departments of Obstetrics and Gynaecology and ³Paediatrics University of Aarhus ⁴Departments of Paediatrics and ⁵Obstetrics and Gynaecology County Hospital Viborg and the State Serum Institute Copenhagen Denmark

ABSTRACT Kerzel Andersen H Brostrøm Karin Brogård Hansen J Leerhøy J Pedersen M Østerballe O Felsager U and Mogensen S (Institute of Medical Microbiology Departments of Obstetrics and Gynaecology and Paediatrics University of Aarhus Departments of Paediatrics and Obstetrics and Gynaecology County Hospital Viborg and the State Serum Institute Copenhagen Denmark) A prospective study on the incidence and significance of congenital cytomegalovirus infection. *Acta Paediatr Scand* 68 329 1979 — Screening of 3060 neonates for congenital cytomegalovirus (CMV) infection by virus excretion in the urine showed an overall incidence of 0.4%. The incidence was about 1% for mothers between 16 and 25 years and only 0.2% for mothers between 25 and 35. No mothers over 35 years of age gave birth to congenitally infected infants. The percentage of women in the child bearing age susceptible to CMV infection was estimated by the absence of CMV complement fixing antibodies in cord sera and ranged from 48% to 33% with increasing age. None of the infected infants showed obvious signs of congenital CMV infection at birth. At follow up two infants showed slight but transient symptoms compatible with a foetal infection a pair of premature twins exhibited retarded physical and psychomotor development but this could just as well be ascribed to the prematurity itself. None of the infants had detectable CMV-IgM antibodies in cord sera but a trend towards elevated total IgM concentration in cord sera and elevated virus excretion titres appeared in the infants with symptoms. With the very low incidence and no signs of sensorimotor sequelae the preliminary conclusion is that foetal CMV infection in our population by no means has a significance to deserve routine screening or a vaccination programme.

KEY WORDS Cytomegalovirus congenital infection

The classical cytomegalic inclusion disease of the newborn caused by cytomegalovirus (CMV) is well known. The clinical picture exhibits a variety of symptoms the best known being those involving the brain and giving rise to microcephalus meningo-encephalitis and cerebral calcifications, ocular lesions characterized by chorioretinitis and optic atrophy, hepatosplenomegaly and haematological disturbances leading to thrombocytopenic purpura and haemolytic anaemia.

Since 1946 it has been possible to diagnose the infection *intra vitam* and 10 years later Hanshaw stated after an investigation of 17 cases that congenital infection was invariably associated with disease and a poor prognosis especially as regards future psychomotor de-

velopment (9). However during recent years several investigations on the incidence and significance of congenital CMV infection have shown examples with slight and varied clinical manifestations as well as cases with no signs of overt disease at all (12, 17, 19, 21, 22). In these studies the symptomatology and incidence vary widely according to differences in the populations investigated and as to whether the diagnosis was established by virus isolation or antibody determinations (13, 15, 18). Therefore it seems important to perform a study of congenital CMV infection in a Scandinavian population especially as prophylaxis of the infection with a vaccine may become possible although the vaccine has not yet been sufficiently tested (6, 16).

Details of social reasons were given in the article concerning 4 of 13 cases. As most of the others were hospitalised for less than five days it is unlikely that they were all seriously ill and still could leave the hospital after only a few days.

I agree with Dr Eriksson that good nursing care is of value in many cases but however good nursing care may be at a modern hospital it could not possibly have influenced the course of the disease of the children who were never hospitalised (35 of 59) and again I want to stress that the majority of the hospitalised children left hospital after less than five days. I agree with Dr Eriksson that if possible

small children should be protected from whooping cough but erythromycin treatment should be used with some restriction as if too widely used resistant strains may appear. Erythromycin can also cause gastrointestinal side effects and a child with whooping cough is not much helped by also having diarrhoea.

Finally I think the most important finding in my study was that whooping cough even in the smallest children can have a very variable clinical course from serious cases needing assisted ventilation to very mild cases with nonspecific cough and good general condition.

B Trollfors

Table 1 *The incidence of congenital CMV infection in different age groups of mothers*

Age group (years)	Study groups				Mothers with infected infant		
	University Hospital		County Hospital		Total No	No	Incidence at age group (%)
	No	%	No	%			
16-19	51	2.5	48	4.6	99	1	1.0
20-4	418	0.7	302	28.9	720	7	1.0
25-9	830	4.1	475	40.8	1255	2	0.2
30-34	499	24.7	183	17.6	682	1	0.2
35-40	174	8.6	76	7.3	250	0	0
≥40	45		9	0.9	54	0	0
Total	2017		1043		3060	11	0.4

One mother gave birth to a pair of infected twins

haemolysis, thrombocytopenia and hepatobiliary involvement but only infected infants were subjected to ophthalmological examination and X-ray of the skull, chest and extremities.

RESULTS

The incidence of congenital CMV infection

A total number of 478 pools of unnes from the neonates of 2017 deliveries (30 pairs of twins) at the University Hospital and 366 pools from 1043 deliveries (4 pairs of twins) at the County Hospital were cultured for CMV. A total of 10 pools seven from the University Hospital and 3 from the County Hospital yielded CMV whereas no other viruses were detected. By examination of the individually stored unnes the excreting infants were easily found in all cases except one. In this case the excreting infant was found after reexamination attempts

from the respective infants at 7 weeks of age. In the 8 pools of CMV containing unnes only one infant was found to be the excreter whereas in two pools two unrelated infants and two twins respectively were found to be infected. By using this method the diagnosis was established between 14 and 26 days after the arrival of the specimen except in the case in which new specimens had to be collected.

Thus 8 infants from 7 deliveries (0.4%) at the University Hospital and 4 infants from the County Hospital (0.4%) were found to be infected at birth. The incidence in relation to the age of the mothers is shown in Table 1. It is relatively high (1%) in the age groups under 25 years and much lower (0.2%) in the age groups between 25 and 35. This difference is statistically significant ($P < 0.01$). None of the mothers over 35 years delivered congenitally infected infants. No difference concern

Table 2 *Number of CMV seropositive individuals in different age groups*

Age groups (years)	Mothers ^a from					
	Female blood donors		University Hospital		County Hospital	
	No	%	No	%	No	%
16-4	40/66	61	35/66	53	51/99	52
5-9	51/90	57	77/137	46	68/178	53
10-14	6/39	67	4/67	67	35/60	58
15-19	13/17	76	16/54	64	1/18	67
20-24	13/17	76	5/6		1/1	
Total	143/179	80	175/196	49	167/307	54

^a Hospital staff

The serological findings in mothers are based on studies of cord sera from their infants.

For this purpose 3000 infants born in two Danish hospitals were screened for urinary CMV excretion at birth and infected infants were followed up to in age ranging from 8 months to 2 years.

MATERIALS AND METHODS

Study groups The study included infants born at the County Hospital of Viborg a general hospital which accepts all deliveries in that county and infants born at the Department of Obstetrics and Gynaecology University Hospital Aarhus. The University Hospital receives mostly women with pathological and potentially pathological pregnancies and deliveries.

The study from the County Hospital covered the period from November 1974 through October 1975 and 1047 of 1109 neonates (94%) were tested. The study from the University Hospital ran from March 1976 through February 1977 and included 2047 of 2460 infants (83%). It was planned to collect urine from all neonates during their one week stay in the hospital but isolation attempts had to be stopped during Easter and Christmas. Therefore infants born during these periods were excluded and so were some other infants because of unsuccessful collection or urine isolation attempts from 67 unnes failed in the laboratory because of broken glasses or contamination of the cultures with bacteria or fungi.

Furthermore material from 36 stillborn or perinatally dead infants were included in the study. This constitutes about one half of the total number of cases the other half being excluded because of postmortal autolysis.

Specimens Urine from infants was collected in plastic urinary bags during the first 5 days of life and kept at 4-10°C until transport to the laboratory. From the County Hospital the specimens were posted overnight in containers at 0-10°C (Nunc) whereas the material from the University Hospital arrived in the laboratory within one hour. The hospitals were instructed never to freeze the specimens. The dead infants were autopsied and small specimens of the lung, liver and kidney were cultured for virus. When possible blood from the umbilical cord was drawn for serum antibody study.

Virus isolation procedure Monolayers of human embryonic lung fibroblasts in 700 ml bottles were used for virus isolation as described previously (2). Urine was mixed with equal parts of Eagle minimum essential medium (MEM) with 10% calf serum and adjusted to pH 7.2 with a 2.8% solution of sodium bicarbonate and stored in that condition for one month at 4°C. For the screening procedure 1 ml of pH adjusted unnes from 2-5 infants were pooled and added to a tissue culture for 1 hour at 37°C with tilting of the bottle every 15 min. After the adsorption the cultures were overlaid with MEM containing 2% calf serum and antibiotics (gentamicin and mycostatin) for 2 days after which the medium was changed to Dulbecco's special medium with 2% serum and antibiotics. Cultures were observed during the first few days for toxic effect of the unnes and if necessary the medium was changed or the amount of serum

was increased for a few days to keep the cells in good condition. The medium was changed once a week and the cultures were observed for cytopathic effect twice a week for a total of 4 weeks. Cultures which were negative for virus growth after more than 20 days of culture but were lost during the fourth week were considered negative and included in the material. Individual cultures for virus were made of stored unnes from pools yielding growth of CMV. Later samples from virus excretors were transported in 2 ml of MEM + 10% calf serum. Autopsy tissue was squeezed and added to a culture for 3 hours after which the culture medium was changed.

Viruses were identified as CMV by their slowly developing cytopathic effect in tissue culture and the formation of small plaques stainable with methylene blue. The number of plaques were counted before secondary plaques had formed and on the basis of this the virus excretion titre was estimated.

Check of the isolation procedure The transport system and the sensitivity of the culture procedure were controlled 25 times during the study. Urine specimens from 6 known CMV-excreting infants were posted overnight like the urine specimens from the County Hospital and cultured for virus. As CMV was isolated in each instance the procedure was regarded as sufficient.

Serological studies The Ad 169 strain was employed as the source of antigen for all serological procedures. Complement fixing (CF) antibodies were determined by a micromethod as described earlier (2).

Levels of IgM in cord sera were measured by immunodiffusion on plates from Behringwerke AG, Germany. All serum samples from congenitally infected infants and controls were investigated simultaneously and levels < 18 mg/100 ml were considered as normal.

Studies of specific CMV IgM antibodies using an immunofluorescence test were done as previously described (3). The sera were tested in dilutions from 1:10 with known positive and negative controls.

Clinical assessment Most of the infected infants from the University Hospital were born after a more or less complicated pregnancy and/or delivery. A control group was selected from the same department. The next two infants of the same sex and birth weight ± 100 g and with the mothers age matched were selected. Examination took place at 8 weeks and 8 months of age. No controls were available for the pair of infected premature twins. Infected infants from the County Hospital the mothers of whom comprised a general delivery population were evaluated without controls as soon as the diagnosis was established and every third month thereafter until the age of 2 years.

Records of the pregnancy, delivery and neonatal period were scrutinized retrospectively with respect to risk factors as maternal abnormalities and pregnancy and birth complications and clinical signs of CMV infection.

The clinical assessment consisted of a complete physical and neurological examination. The infants were not exposed to a refined infant intelligence test but milestones for gross sensorimotor development were used in the judgement of mental and motor capabilities.

At the first visit a complete blood count and blood chemistry determination were performed for signs of

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16-19	51	2.5	48	4.6	99	1	1.0
20-24	418	20.7	307	8.9	725	7	1.0
25-29	830	41.2	475	40.8	1305	2	0.2
30-34	499	24.7	183	17.6	682	1	0.2
35-40	174	8.6	76	7.3	250	0	0
≥40	45	2.2	9	0.9	54	0	0
Total	2017		1043		3060	11	0.4

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Table 2 *Number of CMV seropositive individuals in different age groups*

Age groups (years)	Female blood donors		Mothers* from			
			University Hospital		County Hospital	
	No	%	No	%	No	%
16-19	40/66	61	35/66	53	51/99	57
20-24	51/90	57	77/137	56	68/118	53
25-29	6/39	67	4/16	67	35/60	58
30-34	13/17	76	16/15	63	1/18	67
35-44	13/17	76	5/6	63	1/2	
Total	143/199	72	175/196	59	167/307	54

*Hospital staff

The serological findings in mothers are based on studies of cord sera from their infants.

Table 3 The main features of the 12 infected infants

Case No	Birth weight and length (g/cm)	Clinical data		Virus excretion titre ($\times 10^3$ PFU/ml)		
		Neonatal period	At follow up	1 week	2 months	8 months
1	3 650/50	Jaundice	Asymptomatic	2.5	2.5	0.1
2	4 000/53	Haemangioma	Asymptomatic	2.0	4.0	0.05
3	3 180/51	Jaundice IgM 27 mg/100 ml	Non specific osseous changes reticulocytosis		12.5	7.0
4	3 700/52	Normal IgM 100 mg/100 ml	Liver and spleen enlargement specific osseous changes reticulocytosis	15		12.5
5	3 960/50	Haemangioma	Head circumference +2 S.D. to length ^b	15	5	7.5
6	2 920/50	Small for date	Specific osseous changes	15	11	7.0
7	1 000/37	Gemellus jaundice IgM 30 mg/100 ml	Retarded psychomotor development mild spastic diplegia failure to thrive deficient rearing ^b	≥ 100		≥ 100
8	1 380/41	Gemellus jaundice IgM 60 mg/100 ml	Retarded psychomotor development failure to thrive deficient rearing ^b	≥ 100		12.5
9	2 800/49	Small for date jittering and hyperexcitability	Asymptomatic			
10	2 450/49	Small for date	Asymptomatic			
11	4 200/55	Normal	Asymptomatic			
12	3 400/51	Normal	Asymptomatic			

Normalized at 8 months

^b Unchanged at 8 months

ing the incidence in relation to the groups was found between the two study groups neither was any seasonal variation noticed. All mothers except one were primipara. Virus was not isolated from any of the dead infants. CMV excretion was continued in all infected infants. Table 3 shows the virus excretion titres of the infected infants from the University Hospital. The first two cases were characterized by fairly low and decreasing titres whereas the next four showed about 10 times higher levels of CMV units. The twins excreted about 10^4 plaque forming units/ml and this high level persisted in one whereas a 10 fold decrease was seen in the other during the follow up. Throat swabs from the congenitally infected infants were positive in all but two cases.

Serological studies Since CMV antibodies are transferred transplacentally the degree of previous exposure of the mothers to the virus was evaluated by examining about 300 randomly taken cord sera from each hospital group for CF antibodies to CMV. The results were compared with the degree of seropositivity found in a group of female blood donors from the hospital staff. As seen from Table 4 the mean rate of seropositivity varies between 54 and 62% and a typical rise in seropositivity is seen with increasing age in all three groups.

Cord sera from 11 of the infected infants and from the 12 control infants were available for determination of IgM concentration. All control infants and all but 4 of the infected infants showed levels of IgM less than 18 mg/100 ml. The infected infants with elevated cor-

centration of IgM were cases Nos. 3 and 4 and the pair of twins (Table 3). It was however not possible to demonstrate specific CMV IgM antibodies in any of these sera.

Clinical findings Table 3 summarizes the main features of the 12 infected infants in the study. Evaluation of the six of the eight infected infants from the University Hospital (cases 1-6) and of the 12 controls showed that risk factors in respect to pregnancy and delivery were comparable. None of the infected infants showed obvious symptoms suggestive of CMV infection. Mean Apgar scores at 1 min were 9.5 and 9.2 respectively with the highest score in the infected group. Two of the infected infants were jaundiced while only one of the controls exhibited significant hyperbilirubinaemia. No infants in either group showed major congenital defects but two infected infants were born with small haemangiomas of the strawberry type. Both infected and control infants were discharged after a mean period of 6.3 days but mean weight losses from birth to the fifth day of life was greater in the infected group (mean 183 g S.D. 116 g vs. 96 g S.D. 180 g $P=0.15$).

At 8 weeks and 8 months of age all infants were in good health but physical examination at the first visit revealed in one viruric infant enlargement of the liver and spleen which were 2 cm below the costal margins. The two haemangiomas were regressing normally. Growth and nutritional status including weights of all infants were within normal limits. The head circumference of one viruric infant differed -2.8 D to length on both visits though she had no microcephalic look. The head circumference of all other infants were appropriate to length centiles. No neurological defects or hearing impairments were found and the psychomotor development at 8 months of age was within normal limits in all cases.

The two remaining patients from the University Hospital fraternal twins (cases 7-8) were born of a 22 year-old Turkish mother. They were nursed in incubators for one month

but only one week of oxygen supply was necessary. Except for slight hyperbilirubinaemia they behaved well and there were no specific signs of systemic CMV infection. Discharge took place to an infant home because of bad parental and social circumstances but from 9 months of age they were reared at home. Their physical and psychomotor development was moderately retarded at follow up at 5 and 11 months of age.

Of the 4 infants from the County Hospital (cases 9-12) two were born small for gestational ages according to standard criteria. The neonatal period gave no suspicion of CMV infection. One of the infants born small for gestational age was born after vacuum extraction because of hypertension *sub partu*. This infant was jittering and showed hyperexcitability in the first few weeks; the others behaved normally. All 4 infants followed to the age of 2 years were normal as regard physical examination, psychomotor development and hearing.

Laboratory investigations as measured at the first examination None of the infants had thrombocytopenia. There were signs of overt haemolysis measured by haemoglobin, MCV, reticulocytes and serum bilirubin although two viruric infants exhibited the highest reticulocyte counts of 4.8% and 4.2% (infected mean 2.3% S.D. 2.0% and control mean 1.5% S.D. 1.0% not significant). Mean total lymphocyte counts determined from the total white blood count multiplied by the per cent of lymphocytes on differential white cell count were significantly higher in the infected group (mean $9.56 \times 10^9/l$ S.D. $2.00 \times 10^9/l$ and $6.54 \times 10^9/l$ S.D. $2.50 \times 10^9/l$ $P=0.01$). No hepatobiliary involvement was present as judged by serum GOT and alkaline phosphatase. Urine analyses for albumin, reducing substances and pyuria were unremarkable.

Ophthalmological and X-ray examinations Ophthalmological examination in the 12 infected infants revealed no visual disturbances, microphthalmos or chorioretinitis. X-ray examination of the skull showed no cerebral cal

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Cord sera from 11 of the infected infants and from the 12 control infants were available for determination of IgM concentration. All control infants and all but 4 of the infected infants showed levels of IgM less than 18 mg/100 ml. The infected infants with elevated con-

These latter diseases were not probable in our cases as the infants were seronegative for IgM rubella antibodies and the mothers were WR negative. The prognostic value of determining virus excretion titres is uncertain. There are differences between the infants both in the initial titres and the titres at follow up. However, later examination of the children is needed to draw any conclusions as to the significance of this parameter. Melish & Hanshaw (13) found a correlation between the presence of specific CMV IgM antibodies and severe systemic damage. Four of our infants had elevated levels of IgM antibodies but none had specific CMV IgM antibodies at birth.

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REFERENCES

1. Altschuler G & McAdams A J. Cytomegalic inclusion disease of a nineteen week fetus. *Am J Obstet Gynecol* 111: 795, 1975.
2. Andersen H K & Spencer E S. Cytomegalovirus infection among renal allograft recipients. *Acta Med Scand* 186: 7, 1969.
3. Andersen P & Andersen H K. Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in cytomegalovirus infection. *J Clin Lab Immunol* 1979, in press.
4. Berenberg W & Nankervis G. Long term follow up of cytomegalic inclusion disease of infancy. *Pediatrics* 46: 403, 1970.
5. Collaborative study. Cytomegalovirus infection in the North West of England. *Arch Dis Child* 43: 313, 1970.
6. Elek M D & Siem H. Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. *Lancet* i: 1, 1974.
7. Embel I A, Otter W L & Haldane E V. Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *J Pediatr* 77: 417, 1970.
8. Graham C B, Thal A & Wassum C S. Rubella like bone changes in congenital cytomegalic inclusion disease. *Radiology* 94: 39, 1970.
9. Hanshaw J H. Congenital and acquired cytomegalovirus infection. *Pediatr Clin North Am* 13: 279, 1966.
10. Kirsch U. Complement fixing antibodies against cytomegalovirus in different parts of the world. *Bull Wild Hlth Org* 49: 103, 1973.
11. Kirsch U, Konayev Z & Jung M. Congenital cytomegalovirus infection in siblings from consecutive pregnancies. *Helv Paediatr Acta* 26: 355, 1971.
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REFERENCES

1. Altschuler G & McAdams A J. Cytomegalic inclusion disease of a nineteen week fetus. *Am J Obstet Gynecol* 115: 295 1971.
2. Andersen H A & Spencer E S. Cytomegalovirus infection among renal allograft recipients. *Acta Med Scand* 186: 7 1969.
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5. Collaborative study. Cytomegalovirus infection in the North West of England. *Arch Dis Child* 43: 313 1970.
6. Elek S D & Stern H. Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. *Lancet* i: 1 1974.
7. Embil S A, Ozert R L & Haidons E V. Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *J Pediatr* 77: 417 1970.
8. Graham C B, Thal A & Wassum C S. Rubella like bone changes in congenital cytomegalic inclusion disease. *Radiology* 94: 19 1970.
9. Hanshaw J H. Congenital and acquired cytomegalovirus infection. *Pediatr Clin North Am* 13: 279 1966.
10. Krech U. Complement fixing antibodies against cytomegalovirus in different parts of the world. *Bull Wild Hlth Org* 49: 103 1973.
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REFERENCES

1. Altschuler G. & McAdams A. J. Cytomegalic inclusion disease of a nineteen week fetus. *Am J Obstet Gynecol* 111: 795, 1971.
2. Andersen H. K. & Spencer E. S. Cytomegalovirus infection among renal allograft recipients. *Acta Med Scand* 186: 7, 1969.
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5. Collaborative study. Cytomegalovirus infection in the North West of England. *Arch Dis Child* 45: 513, 1970.
6. Elek S. D. & Stern H. Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. *Lancet* i: 1, 1974.
7. Embil J. A., Ozere R. L. & Haldane E. V. Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *J Pediatr* 77: 417, 1970.
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9. Hanshaw J. B. Congenital and acquired cytomegalovirus infection. *Pediatr Clin North Am* 13: 279, 1966.
10. Krech U. Complement fixing antibodies against cytomegalovirus in different parts of the world. *Br J Med Hyg* 49: 103, 1973.
11. Krech U., Konjajev Z. & Jung M. Congenital cytomegalovirus infection in siblings from consecutive pregnancies. *Helv Paediatr Acta* 26: 355, 1971.
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REFERENCES

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9. Hanshaw J. B. Congenital and acquired cytomegalovirus infection. *Pediatr Clin North Am* 13: 779, 1966.
10. Krech U. Complement fixing antibodies against cytomegalovirus in different parts of the world. *Bull Wild Hlth Org* 49: 103, 1973.
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REFERENCES

1. Altschuler G & McAdams A J. Cytomegalic inclusion disease of a nineteen week fetus. *Am J Obstet Gynecol* 111: 495, 1971.
2. Andersen H K & Spencer E S. Cytomegalovirus infection among renal allograft recipients. *Acta Med Scand* 186: 7, 1969.
3. Andersen P & Andersen H K. Immunoglobulin levels and specific viral antibodies in relation to smooth muscle antibodies in cytomegalovirus infection. *J Clin Lab Immunol* 1979, in press.
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6. Elek S D & Stern H. Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. *Lancet* i: 1, 1974.
7. Embil J A, Ozere M L & Haldane E V. Congenital cytomegalovirus infection in two siblings from consecutive pregnancies. *J Pediatr* 77: 417, 1970.
8. Graham C B, Thal A & Wassum C E. Rubella like bone changes in congenital cytomegalic inclusion disease. *Radiology* 94: 39, 1970.
9. Hanshaw J H. Congenital and acquired cytomegalovirus infection. *Pediatr Clin North Am* 13: 279, 1966.
10. Krech U. Complement fixing antibodies against cytomegalovirus in different parts of the world. *Bull Wild Hlth Org* 49: 103, 1973.
11. Krech U, Konjajev Z & Jung M. Congenital cytomegalovirus infection in siblings from consecutive pregnancies. *Helv Paediatr Acta* 26: 355, 1971.
12. Kumar M L, Nankervis G A & Gold E. Inapparent congenital cytomegalovirus infection. *N Engl J Med* 288: 1370, 1973.

- 13 Melish F M & Hanshaw J B Congenital cytomegalovirus infection: Developmental progress of infants detected by routine screening *Am J Dis Child* 126 190 1973
- 14 Monif R G Fegan E A Held B & Fitzman D V The correlation of maternal cytomegalovirus infection during varying stages in gestation with neonatal involvement *J Pediatr* 80 17 1972
- 15 Nakao T Chiba S Ohsaki M & Ikeda S Studies of cytomegalovirus infection in newborn infants *Tohoku J Exp Med* 105 191 1971
- 16 Plotkin S A Just M & Furquhar J Clinical trials of immunization with live human cytomegalovirus (Towne strain) *Pediatr Res* 10 392 1976
- 17 Reynolds D W Stagno S Stubbs K G Dahle A J Livingston M M Saxon S S & Alford C A Inapparent congenital cytomegalovirus infection with elevated cord IgM levels *N Engl J Med* 290 291 1974
- 18 Silva I M de Kampfner G L Lister C M & Tobin J O H Identification of pregnancies at risk from cytomegalovirus infection *J Hyg Camb* 79 347 1977
- 19 Stagno S Reynolds D W Huang E S Thimes S E Smith R J & Alford C A Congenital cytomegalovirus infection: Occurrence in an immune population *N Engl J Med* 296 1254 1977
- 20 Starr J G & Gold E Screening of newborn infants for cytomegalovirus infection *J Pediatr* 73 820 1968
- 21 Starr J G Barr R D & Gold E Inapparent congenital cytomegalovirus infection: Clinical and epidemiological characteristics in early infancy *N Engl J Med* 283 1075 1970
- 22 Stern H & Tucker S M Prospective study of cytomegalovirus infection in pregnancy *Brit Med J* 2 268 1973
- 23 Yeager A S Martin H P & Stewart J A Congenital cytomegalovirus infection: outcome for the subsequent sibling *Clin Pediatr* 16 45 1977

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NEONATAL SEPTICEMIA AND PERINATAL RISK FACTORS

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ABSTRACT Bergqvist G, Eriksson M and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran's Children's Hospital Stockholm Sweden) Neonatal septicemia and perinatal risk factors. *Acta Paediatr Scand* 68 337 1979. —Many methods for screening and prediction of neonatal septicemia have been tried. In this study a score related to both perinatal risk factors and neonatal diseases was tested upon healthy newborn infants, infants with septicemia and infants with other diseases. Statistical differences were found between infants with neonatal septicemia and infants with other neonatal diseases as well as normal newborns. It was also possible to find a relationship between certain predisposing factors and predominance of certain pathogens. Complications during pregnancy and delivery were most often found in the group B streptococcal combinations of invasive procedures and neonatal diseases in the staphylococcal group and surgical procedures in the gram negative group.

KEY WORDS Newborn septicemia, group B streptococcus, gram negative bacteria, staphylococcus.

Neonatal septicemia is still a serious condition with attendant high mortality (1-4-5). In numerous retrospective studies certain predisposing factors related to pregnancy, delivery as well as neonatal diseases have been identified (4-14). In recent years attention has also been drawn to the risk of cross infections in neonatal intensive care units (6-7-11).

In order to make the earliest possible diagnosis in neonatal septicemia several screening procedures have been tried, often in combination with cultures from various sites (8-9-13). In one study on infants born following premature rupture of the membranes it was shown that babies with high risk scores related to pregnancy and delivery ran a significantly greater risk of developing septicemia (13).

However, no studies have attempted to combine the scores of risk factors related to pregnancy, delivery and procedures used in the care of the infant and relate them to the different causative organisms.

This study was undertaken to evaluate a scoring method in different groups of newborn

infants. Moreover the scores were related to the causative organism found in those infants having neonatal septicemia.

MATERIALS AND METHODS

One hundred and five consecutive newborn infants examined in the maternity ward and 49 consecutive babies admitted to a neonatal unit under different diagnoses were scored. The scores were compared with those of 61 consecutive infants less than 7 weeks of age, cared for at St Goran's Children's Hospital who had neonatal septicemia as evidenced by clinical symptoms and at least one positive blood culture obtained from a peripheral vein. Of these 61 infants, 18 were surgical patients. The infants with neonatal septicemia were born during the years 1970-73 and the other babies in 1973. The incidence of neonatal septicemia has been calculated as 1.4 per 1000 newborns. The infants were given a score of 1 for each of the following 10 items: maternal disease, e.g. diabetes, severe toxemia, infection, rupture of the membranes more than 24 hours before the baby was born, foul smelling amniotic fluid, complicated delivery, e.g. emergency forceps, vacuum extraction and section, preterm birth (gest. age <37 weeks), small for gestational age, Apgar score <7 at 1 minute, umbilical catheterization, respiratory distress and other neonatal diagnoses, e.g. congenital malformation leading to operative procedures and later long periods of i.v. fluid nutrition.

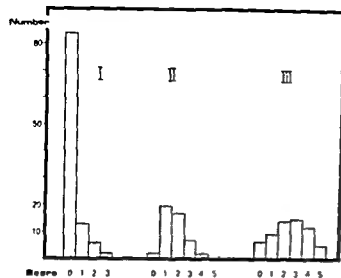


Fig. 1 Neonatal score rates in the three groups of infants which have been studied I 105 consecutive newborn infants II 49 newborn infants consecutively admitted to a pediatric department III 61 cases with neonatal septicemia

For these infants the mother's breast milk or pasteurized milk from other mothers has been the rule except for very sick infants and infants with intestinal malformations where i.v. fluid and parenteral nutrition were given.

For statistical analysis the Kruskal & Wallis multi-sample test was used (3).

In 133 consecutive infants with neonatal septicemia (including the 61 infants that were scored) the total number of perinatal risk factors were grouped according to the four causative organisms: *Staphylococcus aureus* group B streptococcus *E. coli* and gram negative rods other than *E. coli*.

RESULTS

The scoring profiles of the different groups are shown in Fig. 1. There is a significant difference between infants with septicemia and other infants admitted to the neonatal unit. The relation between the number of perinatal risk factors and the causative organism is shown in Table 1. As can be seen a combination of several risk factors related to pregnancy, delivery and procedures performed before the diagnosis is commonly associated with three of the causative organisms while complications during pregnancy and delivery are more often associated with infections caused by group B streptococci. In cases in which there is only one factor related to a procedure before the diagnosis, exchange transfu-

sion dominates in the staphylococcal group and surgical procedures in the gram negative group. Umbilical vessel catheters are common in the staphylococcal and gram negative groups.

DISCUSSION

This study shows that infants who develop septicemia usually have a combination of several predisposing factors. They have a significantly higher score than normal newborns and sick newborns who do not develop septicemia. A score of 3 or more was found in 52% of the total septicemia group but in only 20% of the newborns admitted to a neonatal ward and in 2% of consecutive healthy newborns. With a slightly different scoring system putting more emphasis on pregnancy and delivery, Takkur et al. (13) found that the risk of developing neonatal septicemia rose from 20% with a score of 4 up to 60% with a score of 6. As in our study they too found a few infants with low scores who later developed septicemia.

Some differences were noted on analysis of the relation between the predisposing factors and the causative organisms. Risk factors related to pregnancy and delivery such as maternal infection and premature rupture of the membrane were common in group B streptococcal infections while additional risk factors were common in gram negative infections. Several other investigators have also shown that especially the early onset type of group B streptococcal disease is combined with complications during pregnancy and delivery such as early rupture of the membranes even though nosocomial spread also seems to exist (2). Differences between streptococcal and other neonatal infections have also recently been reported as regards age at onset and mortality (10).

A combination of several risk factors was most often found in staphylococcal and gram negative infections. The increased incidence of so-called nosocomial infections in neonatal intensive care units has attracted increasing at-

Table 1 Relation between the number of perinatal risk factors and the causative organism in 133 infants with neonatal septicemia

Etiology	Total (n)	Only one factor related to		More than one factor related to			No factor (n)
		A) Pregnancy and Delivery (n)	B) Neonatal procedures before Diagnosis (n)	A) Complications during Pregnancy and Delivery (n)	B) Neonatal procedures before Diagnosis (n)	Combinations of A and B (n)	
<i>Staphylococcus aureus</i>	39	3	5	1	1	23	6
Group B streptococcus	25	13	0	3	0	7	5
<i>E. coli</i>	38	3	6	7	2	14	8
Gram negative organisms other than <i>E. coli</i>	30	0	2	0	4	23	1

tion in recent years and has been shown to be present in 25% of the patients (6-7-11). This could be related both to host factors such as low birth weight and environmental factors. It has also been demonstrated that various changes in the ward routine will affect the predominance of bacteria isolated: discontinuation of bathing in hexachlorophene increased the rate of colonization with staphylococci and procedures to reduce this rate instead increased the incidence of gram negative organisms (7-12).

Beside analysing the host factor of birth weight we have also studied other factors such as invasive procedures and found a relationship to the causative organism: i.e. permanent catheters were more commonly associated with staphylococcal infections and operative procedures and parenteral nutrition with gram negative infections.

Prospective studies will be needed not only on infected infants but also on colonization to confirm the relationship between certain predisposing factors and predominance of certain pathogens that we have found.

REFERENCES

1. Altyrup L C & Andrews B H Neonatal sepsis. *Clin Pediatr* 14: 181 1975.
2. Baker C J Summary of the workshop on perinatal infections due to group B streptococcus. *J Infect Dis* 136: 137 1977.
3. Bradley J V *Distribution-free Statistical Tests*. Prentice Hall, New Jersey 1968, p 19.
4. Davies P A Bacterial infection in the foetus and newborn. *Arch Dis Child* 46: 1 1971.
5. Gotoff S P & Behrman R E Neonatal septicemia. *J Pediatr* 76: 142 1970.
6. Hable K A, Madsen J M, Wheeler D J, Hunt C E & Que P G Klebsiella type 33 septicemia in an infant intensive care unit. *J Pediatr* 80: 970 1972.
7. Hemmung V G, Overall J C & Britt M R Nosocomial infections in a newborn intensive care unit. *N Engl J Med* 294: 1310 1976.
8. Higgs S C, Malan A F & De V Heese H The perinatal infective environment and infants of very low birth weight. *S Afr Med J* 51: 621 1977.
9. Hosmer M E & Sprunt K Screening method for identification of infected infant following premature rupture of maternal membranes. *Pediatrics* 49: 283 1972.
10. Jeffery H, Michelson R, Wigglesworth J S & Davies P A Early neonatal bacteraemia: A comparison of group B streptococcal, other gram positive and gram negative infections. *Arch Dis Child* 52: 183 1977.
11. Morehead C D & Houck M W Epidemiology of pseudomonas infections in a pediatric intensive care unit. *Am J Dis Child* 124: 664 1977.
12. Light J J, Sutherland J M, Cochran L & Sutton J Ecologic relation between staphylococcus aureus and pseudomonas in a nursery population. *N Engl J Med* 278: 143 1968.
13. Takkar P, Bhakoo O N & Narang A Scoring system for the prediction of early neonatal infections. *Indian Pediatr* 11: 597 1975.
14. Tyler C W & Albers W H Obstetric factors related to bacteremia in the newborn infant. *Am J Obstet Gynecol* 94: 970 1966.

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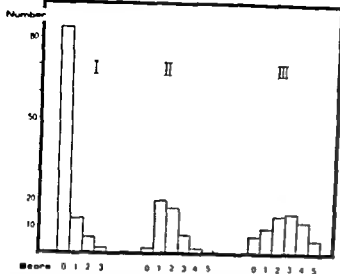


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REFERENCES

1. Akjjan L C & Andrews B F Neonatal sep is *Clin Pediatr* 14 181 1973
2. Baker C J Summary of the workshop on perinatal infections due to group B streptococcus *J Infect Dis* 143 137 1977
3. Bradley J V *Practical Statistical Tests* Prentice Hall, New Jersey 1968 p 149
4. Davies P A Bacterial infection in the foetus and newborn *Arch Dis Child* 46 1 1971
5. Gotoff S P & Behrman R E Neonatal septicemia *J Pediatr* 76 147 1970
6. Hable K A, Maiten J M, Wheeler D J, Hunt C E & Que P G Klebsiella type 33 septicemia in an infant intensive care unit *J Pediatr* 80 970 1971
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8. Higgs S C, Malan A F & De V Heese H The perinatal infective environment and infants of very low birth weight *S Afr Med J* 51 671 1977
9. Hosmer M & Sprunt K Screening method for identification of infected infant following premature rupture of maternal membranes *Pediatrics* 49 783 1972
10. Jeffery H, Mitchison R, Wigglesworth J S & Davies P A Early neonatal bacteraemia. A comparison of group B streptococcal, other gram positive and gram negative infections *Arch Dis Child* 52 683 1977
11. Morehead C D & Houck P W Epidemiology of pseudomonas infections in a pediatric intensive care unit *Am J Dis Child* 124 464 1977
12. Light I J, Sutherland J W, Cochran L & Sutorius J Ecologic relation between staphylococcus aureus and pseudomonas in a nursery population *N Engl J Med* 278 1 43 1968
13. Takkar P, Bhakoo O N & Narang A Scoring system for the prediction of early neonatal infections *Indian Pediatr* 11 597 1975
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DIAGNOSTIC VALUE OF SYMPTOMS AND CLEAN VOIDED URINE SPECIMEN IN CHILDHOOD URINARY TRACT INFECTION

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ABSTRACT Pylkkänen J, Vilksa J and Koskimies O (The Children's Hospital, University of Helsinki, Helsinki, Finland). Diagnostic value of symptoms and clean voided urine specimen in childhood urinary tract infection. *Acta Paediatr Scand* 68:341-344, 1979. — In diagnosing urinary tract infection (UTI) the symptoms of 477 infants and children and the findings in their clean voided urine specimens were evaluated. 322 patients were considered infected when a bacterial culture of suprapubic aspirate was used as a diagnostic reference. No diagnosis was attempted on the basis of symptoms only. Numerous bacteria or ≥ 200 leuc/mm³ in an uncentrifuged clean voided urine specimen or $\geq 10^5$ bact/ml in quantitative bacterial culture were found in 49%, 42% and 81% of the infected symptomatic patients. The diagnostic accuracies of these indices were 88%, 94% and 95%, respectively. In asymptomatic patients the accuracies were considerably lower. Among these infected patients normal or equivocal isolated findings in the clean voided urine specimens were frequently seen and could not markedly be reduced by the various related factors such as technique of urine collection, urine specific gravity or pH of urine. None of the above mentioned indices of the clean voided urine specimens seems to be alone accurate and sensitive enough for diagnosing UTI and therefore these should be used in combination. The advantage of immediately obtaining results supports the use of urine microscopy as a primary diagnostic method in symptomatic UTI of childhood in particular.

KEY WORDS Urinary tract infection, suprapubic bladder aspiration, children.

Suprapubic aspiration of urine (SPA) is the most reliable way to diagnose or exclude childhood urinary tract infection (UTI) (1-4). However, UTI is usually suspected on the basis of its symptoms and diagnosed by analysing clean voided urine specimen (CVUS). We evaluated the symptoms, the counts of leucocytes and bacteria on microscopy and the quantitative bacterial cultures from the uncentrifuged CVUSs and used the bacterial culture from SPA as a diagnostic reference. The effect of the various related factors on the findings in the CVUSs was also investigated.

PATIENTS AND METHODS

477 patients (164 infants and 313 children) seen at the general outpatient clinic were included in the study. On the basis of the findings in their SPA 379 patients (74 infants and 48 children) were shown to have UTI. Of these 477 patients 77 had symptoms suggesting acute

UTI. SPA was also performed on 705 asymptomatic patients because of a high leucocyte count in or an abnormal quantitative bacterial culture from their CVUSs.

Techniques of urine collection. SPA was performed as described by Saccharov & Pyles (8). No complications other than occasional microscopic hematuria were seen. Both CVUS and SPA were always obtained within the same 24-hour period. Before collecting CVUS the external genitalia were gently cleansed with 0.05% Chlorhexidine solution then rinsed several times with water and dried. Coloplast® urine collection bags (Espergærde, Denmark) were used on the infants. The bag was changed every hour until a sample was voided. The collection of mid-stream samples from the children was performed as described by Huttunen et al. (6).

Analysis of the CVUSs. Immediately after collection the well-mixed CVUSs were examined under microscope for leucocyte and bacterial count using a Burkert counting chamber. A sample was also obtained to carry out quantitative bacterial culture (Uncult®; Onco Pharmaceutical Co., Helsinki). The amounts of leucocytes were grouped ≤ 10 , 11-199 or ≥ 200 leuc/mm³. The numbers of bacteria in the native preparation were expressed as nil, scanty or numerous (≥ 30 bact/microscopic field). The bacterial cultures were classified $\leq 10^4$, 10^4 or $\geq 10^5$ bact/ml.

Table 1 Principal complaints suggesting UTI

200 children with suspect first UTI. Every patient may have one or several leading symptoms

Age (years)	<1.0	1.0-2.9	≥3.0
Bacterial culture from SPA	+/-	+/-	+/-
Enuresis	0/0	6/2	4/0
Frequent micturition	3/3	10/7	14/6
Painful micturition	7/5	17/8	23/8
Bad smell of urine	4/0	3/0	0/0
Blood in urine	1/0	5/2	3/0
Vomiting	14/8	4/6	8/7
Abdominal pain	3/0	2/3	24/14
Diarrhoea	6/5	4/0	2/2
Obstipation	2/0	0/1	0/0
Back pain	0/0	1/0	2/1
Convulsions	6/1	1/2	0/2
Fever alone	11/8	2/0	0/1
Number of patients	49/76	30/24	44/23

Diagnosis of UTI. The SPAs were cultured on both Uncult[®] dipsticks and blood agar plates. The patient was considered infected (SPA positive) when both cultures showed consistent bacterial growth. In 6 cases the plates or the slides had 1-2 occasional colonies considered to be due to asexual contamination.

Calculations. The chi-square test was employed in comparing the proportions of the CVUS findings.

RESULTS

Value of symptoms in diagnosing UTI

200 patients were suspected to have their first symptomatic UTI and 127 of them were SPA positive. The principal complaints among the infected and non infected children are listed in Table 1. The same symptoms were found in both groups. The only slight difference was that the 8 patients whose mothers had observed bad smell of urine belonged to the infected group.

CVUS findings in diagnosing UTI

Table 2 shows the results from the CVUSs from the 322 infected and 155 non infected patients. All isolated leucocyte or bacterial findings on microscopy and in the CVUS bacterial cultures were detected in both groups.

Table 3 shows the diagnostic sensitivity

and accuracy of the various indices of the CVUS. Of the symptomatic patients with UTI, 59% had ≥ 200 leuc/mm³. On the other hand, 88% of the children with this finding had UTI. The sensitivity and accuracy figures for numerous bacteria in the native preparations were 42% and 94% and for the bacterial culture of $\geq 10^5$ bact/ml in symptomatic patients 81% and 95% respectively. The corresponding diagnostic accuracies in asymptomatic patients were markedly lower. When the diagnostic limits of the indices were lowered to attain higher sensitivity the accuracies also became poorer.

The leucocyte counts showed no statistically significant relation to the other findings in the CVUSs. Instead, 96% of the patients with numerous bacteria on microscopy had $\geq 10^5$ bact/ml in quantitative urine culture. Of the children with $\leq 10^5$ bact/ml, 48% had numerous bacteria while 15% had no bacteria microscopically.

Effect of various related factors on CVUS findings

Since normal and equivocal findings in the CVUSs were frequently detected in the

Table 2 Findings in uncentrifuged CVUSs: 322 infected (SPA positive) and 155 non infected patients

Bacterial culture from SPA	Symptomatic patients +/-	Asymptomatic patients +/-
Leucocyte count (cells/mm ³)		
≥200	117/16	36/1 [†]
11-199	61/34	57/35
≤10	21/23	35/34
Number of bacteria		
Numerous	83/5	64/6
Scanty	89/70	47/74
Nil	27/48	17/52
Quantitative bacterial culture (bact/ml)		
≥10 ⁵	161/9	109/41
10 ⁴	18/9	6/7
≤10 ³	20/55	8/34
Number of patients	199/73	123/82

Table 3 *Diagnostic sensitivity (S) and accuracy (A) of various isolated findings in uncentrifuged CVUSs*

377 infected (SPA positive) and 155 non infected patients

	Symptomatic patients (N=377)		Asymptomatic patients (N=155)	
	S	A	S	A
Leucocyte count (cells/mm ³)				
≥ 00	59%	88%	49%	75%
≥ 11	89%	78%	79%	65%
Number of bacteria				
Numerous	4%	94%	57%	91%
Scanty or numerous	86%	87%	86%	78%
Quantitative bacterial culture (bact./ml)				
≥ 10 ³	81%	95%	89%	73%
≥ 10	90%	89%	93%	70%

patients with UTI the effect of 5 anamnestic and 4 technical factors on leucocyte count on number of bacteria on microscopy and on quantitative bacterial culture were separately analysed in the infected patients. No significant interaction between the related factors emerged. Only the effects with a possible essential relevance are reported here.

Anamnestic correlations

Sex and age of the patients had no significant effect on the CVUS findings. *Presence or absence of symptoms* (Table 2), the leucocyte counts of the symptomatic patients were higher than those of the asymptomatic children ($p < 0.005$). *Sequence number of UTIs* of the 48 patients with their first symptomatic UTI (age ≥ 3 years) 71% had ≥ 200 leuc./mm³ and in 4% the leucocyte count was normal whereas for the 69 patients with recurrent symptomatic UTI (age ≥ 3 years) these frequencies were 41% and 17% respectively ($p = 0.02$). *Concurrent antibiotic therapy* 19 patients with UTI received antibiotic therapy for upper respiratory infection 15 of them had $\geq 10^3$ bact./ml in the CVUS culture.

Technical correlations

Technique of urine collection under microscope numerous bacteria were found in 29% and no bacteria in 27% of 76 bag specimens from the infected infants while in the mid stream samples this was the case in 51% and 10% of 246 patients ($p < 0.005$). *Urine specific gravity and pH of urine* as well as *sequence number of the CVUSs* had no significant relation to the findings in the CVUSs. Low urine specific gravity (≤ 1.004) and low pH of urine ($\text{pH} \leq 5.0$) were seen in 13% and 5% of the infected patients respectively.

DISCUSSION

The diagnostic accuracy of childhood UTI is apparently far from satisfactory (1, 2). The widely accepted use of urine sediment (3) is one of the causes of confusion pointed out by Houston (5). In order to clarify the diagnostic criteria of UTI we compared the symptoms and the findings of uncentrifuged CVUS with the bacterial culture from SPA.

Certain symptoms are thought to suggest UTI in children. In this study the infected and non infected patients could not be discriminated on the basis of their symptoms. 73 of the 200 children with suspect symptomatic UTI had sterile SPASs. Thus a reliable diagnosis of UTI cannot be made without urine analysis.

In a typical case of UTI numerous bacteria and ≥ 200 leuc./mm³ are microscopically detected in uncentrifuged CVUSs and $\geq 10^3$ bact./ml are found in urine culture. Nevertheless each one of these isolated findings was frequently seen in the urine of non infected patients. Equivocal or even normal findings were not uncommon among the infected patients e.g. low leucocyte counts were especially found in recurrent and asymptomatic UTIs. These findings did not show any distinct relation to the various technical factors such as technique of urine collection, urine specific gravity and pH of urine.

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RESULTS

Value of symptoms in diagnosing UTI

200 patients were suspected to have their first symptomatic UTI and 127 of them were SPA positive. The principal complaints among the infected and non infected children are listed in Table 1. The same symptoms were found in both groups. The only slight difference was that the 8 patients whose mothers had observed bad smell of urine belonged to the infected group.

CVUS findings in diagnosing UTI

Table 2 shows the results from the CVUSs from the 322 infected and 155 non infected patients. All isolated leucocyte or bacterial findings on microscopy and in the CVUS bacterial cultures were detected in both groups.

Table 3 shows the diagnostic sensitivity

and accuracy of the various indices of the CVUS. Of the symptomatic patients with UTI 59% had ≥ 200 leuc/mm³. On the other hand 88% of the children with this finding had UTI. The sensitivity and accuracy figures for numerous bacteria in the native preparations were 42% and 94% and for the bacterial culture of $\geq 10^5$ bact/ml in symptomatic patients 81% and 95% respectively. The corresponding diagnostic accuracies in asymptomatic patients were markedly lower. When the diagnostic limits of the indices were lowered to attain higher sensitivity the accuracies also became poorer.

The leucocyte counts showed no statistically significant relation to the other findings in the CVUSs. Instead 96% of the patients with numerous bacteria on microscopy had $\geq 10^5$ bact/ml in quantitative urine culture. Of the children with $\leq 10^3$ bact/ml 48% had numerous bacteria while 15% had no bacteria microscopically.

Effect of various related factors on CVUS findings

Since normal and equivocal findings in the CVUSs were frequently detected in the

Table 2 Findings in uncentrifuged CVUSs: 322 infected (SPA positive) and 155 non infected patients

Bacterial culture from SPA	Symptomatic patients +/-	Asymptomatic patients +/-
Leucocyte count (cells/mm ³)		
≥200	117/16	36/19
11-199	61/34	52/35
≤10	21/73	35/35
Number of bacteria		
Numerous	83/5	64/6
Scanty	89/70	42/74
Nil	27/48	17/57
Quantitative bacterial culture (bact/ml)		
≥10 ⁵	161/9	109/41
10 ⁴	18/9	6/7
≤10 ³	20/55	8/34
Number of patients	199/73	123/87

MITRAL AND TRICUSPID VALVE VEGETATIONS IN INFANCY DIAGNOSED BY ECHOCARDIOGRAPHY

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ABSTRACT Lundström N R and Björkhem G (Department of Paediatrics, University Hospital, Lund, Sweden). Mitral and tricuspid valve vegetations in infancy diagnosed by echocardiography. *Acta Paediatr Scand* 68:345, 1979.—The use of echocardiography in diagnosing intra-cardiac vegetations is described in two infants. The condition is unusual in infancy but can be suspected from the clinical picture and positive blood cultures. Using echocardiography it is possible to localize the vegetations exactly and estimate the extent of valvular involvement. In one of the cases presented a vegetation on the anterior mitral leaflet was diagnosed and a gradual increase in size could be demonstrated. In the other case a large echo-producing mass was found attached to the tricuspid valve protruding into the right ventricular outflow tract. This mass represented a fungal vegetation. In both cases autopsy later verified the echocardiographic findings.

KEY WORDS Two-dimensional echocardiography, cross-sectional echocardiography, bacterial endocarditis, fungal endocarditis.

The diagnosis of bacterial or fungal endocarditis is usually based on clinical findings combined with positive blood cultures. At autopsy one main finding is bacterial or fungal valvular vegetations. Echocardiography has already been shown to be useful in demonstrating the valvular vegetations in infective endocarditis (4, 6, 8, 11, 12, 14, 17). The condition is unusual in infancy (10) and we report here 2 infants in whom echocardiography demonstrated vegetations on the mitral and tricuspid valves respectively.

CASE REPORTS

Case 1
A newborn boy was admitted to the department of paediatrics at the age of one day because of vomiting. No signs of intestinal obstruction were found. At the age of one week the patient developed a low grade fever and muscular hypotonia. Two days later he was transferred to the department of paediatrics. On admission a septicaemia was suspected clinically and there were no signs of heart disease. Blood and urine cultures revealed growth of plasma-coagulating staphylococci which was treated with appropriate antibiotics. Within a few days the fever disappeared and the infant was well and blood cultures were negative. Two weeks later the infant

suddenly developed signs of severe congestive heart failure. Auscultation revealed a high frequency pansystolic murmur at the apical region. A chest X-ray revealed left atrial and right ventricular enlargement. Repeat echocardiographic examinations were performed and the results will be reported below. Despite vigorous treatment (antibiotics, digitalis, diuretics) the condition of the patient deteriorated and he died at the age of 6 weeks 11 days after the onset of congestive heart failure.

Autopsy. The heart was grossly dilated but there was no evidence of congenital heart disease. The anterior mitral leaflet was to a large extent destroyed and replaced by a large vegetation 8 mm in diameter. The posterior mitral leaflet was normal.

Echocardiography. The examinations were performed with a standard ultrasonoscope (Ekoline 70 Smith Kline) and a 2.5 MHz unfocused transducer using standard techniques (13). Great care was taken to use the same gain settings at the repeat examinations.

The first echocardiographic examination was performed on the day of the onset of congestive heart failure (Day 1). The echo from the anterior mitral leaflet showed a normal amplitude and pattern of movement but was unusually thick and partly with a fuzzy indistinct appearance (Fig. 1A). The echo from the posterior mitral leaflet was normal. The left atrium and the right ventricle appeared enlarged (Table I).

A repeat echocardiographic examination three days

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We think that none of the indices applied in this study gives alone sufficient sensitivity and accuracy in diagnosing UTI. In particular we observed significant bacteriuria (7) in the CVUS of only 81% of the symptomatic and 89% of the asymptomatic infected patients. In our opinion the use of Chlorhexidine solution did not influence these figures, since the external genitalia were also rinsed with water several times and dried. On the other hand false positive cultures were seen 10% of the symptomatic and even in 50% of the asymptomatic non infected children.

Thus the separate findings of one or even several CVUSs should be combined for the diagnosis and SPA is needed in cases with diagnostic difficulties. On microscopy the presence of numerous bacteria in an uncentrifuged CVUS predicted with great certainty (96%) $\geq 10^5$ bact./ml in the urine culture. This great advantage of immediate results supports the use of urine microscopy as a primary diagnostic method for UTI in severely ill patients in particular. Of course quantitative urine culture is necessary for confirming the diagnosis and for assessing antibiotic sensitivity.

ACKNOWLEDGEMENT

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REFERENCES

- 1 Aronson A S, Gustafson H & Siemmenssen A W. Combined suprapubic aspiration and clean voided urine examination in infants and children. *Acta Paediatr Scand* 67: 396, 1978.
- 2 Bergvall B & Ljungblom B Å. Suprapubic aspiration of the urinary bladder for accurate diagnosis of urinary tract infections in children. *Acta Paediatr Scand* 61: 390, 1972.
- 3 Dolan T F Jr & Meyers A. A survey of management of urinary tract infections in childhood. *Pediatrics* 52: 21, 1973.
- 4 Edelmann C M Jr, Ogwo J E, Fine B P, Martinez A H. The prevalence of bacteriuria in full term and premature newborn infants. *J Paediatr* 87: 125, 1973.
- 5 Houston I H. Measurement of pyuria in urinary tract infections. *Arch Dis Child* 44: 480, 1969.
- 6 Huttunen N P, Mäkelä P & Mella E. Simple method for increasing reliability in diagnosis of urinary infection. *Lancet* i: 22, 1970.
- 7 Kass E H. Asymptomatic infections of the urinary tract. *Trans Am Phys* 69: 96, 1966.
- 8 Saccharow L & Pryles C V. Further experience with the use of percutaneous suprapubic aspiration of the urinary bladder. Bacteriological studies in 6 infants and children. *Pediatrics* 43: 1018, 1969.

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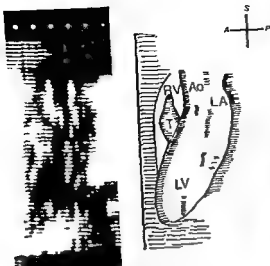


Fig 2 Two-dimensional echocardiogram (still frame from cine film) obtained with the transducer along the long axis of the heart together with schematic drawing showing the tumor mass (T) in the right ventricle (RV) Ao = aortic root LA = left atrium LV = left ventricle A = anterior P = posterior S = superior I = inferior

During three weeks of treatment the boy first improved a little but then slowly deteriorated. Cultures from blood and urine were negative during treatment but since the child did not improve and the echocardiographic examinations still showed the same large mass in the right ventricle surgery was considered necessary. At operation using cardio-pulmonary bypass the right atrium was first opened and a large vegetation was found extending from the base of the septal tricuspid leaflet which was partially destroyed. As much as possible of the mass was removed through the right atrium and the right ventricle was then opened so that the main part of the mass could be reached and extirpated (Fig 3).



Fig 3 Photograph of the candida vegetations removed from the right ventricle during operation on patient no. 7

Postoperatively the child initially did well but after a few hours he developed very low P_{O_2} values in spite of ventilator treatment and died the same evening.

Atopsy. Candida endocarditis was found in the right ventricle with involvement and ulceration of both the septal and the anterior tricuspid leaflets while the posterior leaflet was normal. The left side of the heart was

Table 1 Echocardiographic data obtained at serial examinations in case 1

AM amplitude = amplitude of opening of the echo from the anterior mitral leaflet AM thickness = thickness of the echo from the anterior mitral leaflet LAD/AOD = relation between left atrial dimension (LAD) and aortic root diameter (AOD) LVID = left ventricular internal dimension measured at end-diastole RVD = right ventricular dimension

	AM amplitude (mm)	AM thickness (mm)	Left atrial size (LAD/AOD)	Left ven- tricular size (LVID _{di} mm)	Right ven- tricular size (RVD mm)
Day 1	11	4	1.4	0	1
Day 4	11	4	1.7	1	10
Day 7	10	8	1.35	3	19
Day 9	9	6	1.9	4	16
Day 11	8	7	1.9	8	16
Normal values according to the patient's weight	>6	<6	0.8-1	13-21	4-10

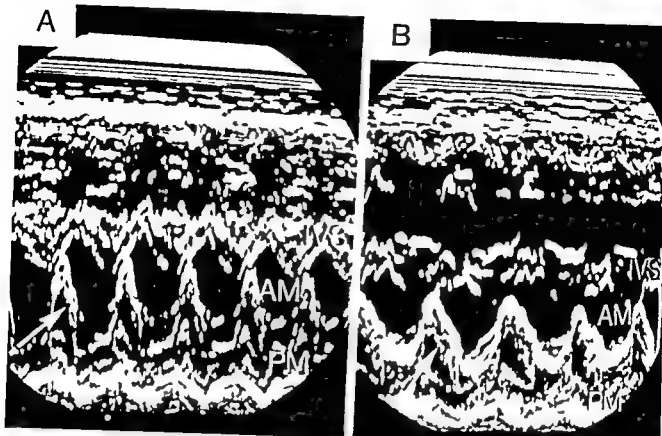


Fig. 1 Echocardiogram obtained from patient no. 1 on day 1 (A) and day 7 (B) with the transducer directed towards the mitral valve. The anterior structures are at the top of the recording. The ultrasonic beam passes the right ventricle, the interventricular septum (IVS) and the left ventricle. In the left ventricle the echoes from

the anterior (AM) and the posterior (PM) leaflets are seen. The echo from the anterior mitral leaflet (arrow) is broader than the echo from the posterior mitral leaflet presumably due to the attached vegetation. On day 7 (B) the echo is still broader than on day 1 (A).

later (Day 4) gave essentially the same results. On day 7 the thickness of the echo from anterior mitral leaflet had increased further (Fig. 1B) and the size of the left and right ventricle had also increased (Table 1). At the last two examinations (Day 9 and 11) the amplitude of movement of the echo from the anterior mitral leaflet had decreased slightly. The thickness of the echo from the anterior mitral leaflet had increased further. The echo from the posterior mitral leaflet was still normal at the last examination. The size of the left atrium and left ventricle had increased further (Table 1).

In summary these echocardiographic examinations showed a gradual increase in thickness of the anterior mitral leaflet, a somewhat reduced amplitude of movement of the anterior mitral leaflet and a normal echo from the posterior mitral leaflet. A gradual increase in size of the left atrium, the left ventricle and the right ventricle could also be demonstrated.

These findings could be explained by a bacterial endocarditis with a vegetation on the anterior mitral leaflet, a severe mitral regurgitation and congestive heart failure.

Case 2

A newborn boy was admitted to the department of pediatric surgery soon after birth because of small urinary volumes and bladder dilatation. A urethral valve

was diagnosed and the child was operated on at the age of five days with bilateral ureterostomy. Postoperatively there was local infection with *Candida albicans*, which was treated with oral cotrimoxazol. One week postoperatively the patient developed signs of urosepsis. Urinary cultures grew *Klebsiella* and the child was treated with antibiotics and improved. At the age of two months the urethral valve was resected and because of urosepsis he was treated with various antibiotics. During the following months his general status deteriorated and several cultures from urine, blood, skin and exudate from the right knee grew *Candida albicans*. Cystoscopy revealed white masses in the bladder representing candida and ophthalmological examination revealed changes in the vitreous body which were suspected to be of candida origin. Cardiac examination at the age of 3.5 months revealed a systolic murmur close to the sternal border with maximal intensity in the fourth intercostal space. A chest X-ray showed moderate cardiomegaly. The electrocardiogram showed signs of right ventricular hypertrophy and right atrial dilatation. Two-dimensional echocardiography revealed a mass in the right ventricle which was interpreted as probably due to candida endocarditis (Fig. 2). The child developed signs of congestive heart failure. He was given intensive antimycotic therapy, digitals and diuretics.

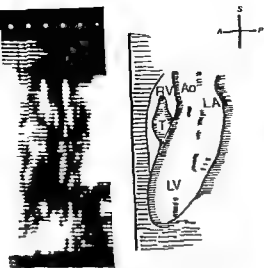


Fig 2 Two-dimensional echocardiogram (still frame from cine film) obtained with the transducer along the long axis of the heart together with schematic drawing showing the tumor mass (T) in the right ventricle (RV). Ao = aortic root LA = left atrium LV = left ventricle A = anterior P = posterior S = superior I = inferior

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	AM amplitude (mm)	AM thickness (mm)	Left atrial size (LAD/AOD)	Left ven- tricular size (LVID _d mm)	Right ven- tricular size (RVD mm)
Day 1	11	4	1.4	0	17
Day 4	11	4	1.2	21	10
Day 7	10	8	1.45	3	19
Day 9	9	6	1.9	4	16
Day 11	8	7	1.9	8	16
Normal value according to the patient's weight	>6	<	0.8-1	13-21	4-10

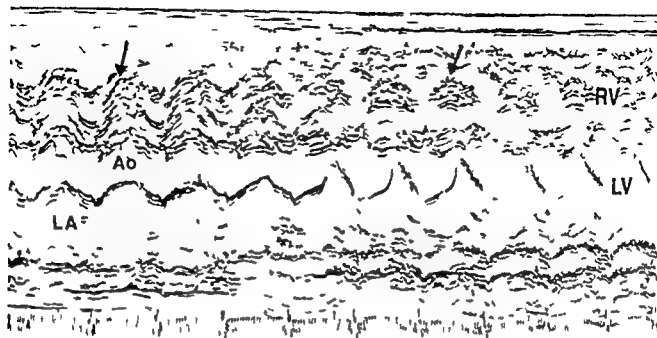


Fig. 4 Echocardiogram from patient no. 2 showing in M mode scan along the long axis of the heart. Arrows indicate the dense mass of echoes found in the right

ventricle (RV) and the right ventricular outflow tract anterior to the aortic root (Ao). LA = left atrium, LV = left ventricle.

not involved. In the main pulmonary artery an adherent candida mass was found attached to the bifurcation almost completely occluding both the left and the right pulmonary arteries. The autopsy also showed bilateral hydronephrosis and a candida abscess in the left kidney.

Echocardiography. The examinations in case 2 were performed with an Echocardiograph ultrasonoscope (Orginson Tekniker) with a fiber optic recorder using a 4.5 MHz unfocused transducer for the single element studies and a 4.5 MHz multi element transducer for the two-dimensional studies. Two-dimensional studies were also performed using an ADR ultrasound real time scanner with a 3.5 MHz transducer. The first examination was performed when the child had developed signs of cardiac involvement at the age of 3.5 months. On the two dimensional image a large echo-producing structure could be seen in the right ventricle (Fig. 2). With multi element and single element technique the structure could be followed from the right ventricle to the level of the tricuspid valve into the right ventricular outflow tract where it had a width of approximately 8 mm (Figs 4-5). On the cross sectional studies the structure appeared to be attached to the base of the septal leaflet and to move into the outflow tract with each heart beat. The single element recordings of the anterior tricuspid leaflet showed multiple echoes during systole while the tip of the leaflet seemed to move freely during diastole. The right atrium appeared to be enlarged while the echocardiographic examinations revealed no abnormalities in the left side of the heart.

The child was followed with repeated examinations up to the time of operation but no decrease in the size of the mass could be shown in spite of the intensive antibiotic treatment.

DISCUSSION

The echocardiographic appearance of the mitral valve vegetation in case no. 1 is similar to that previously described (4, 6, 8, 12, 14). The characteristic feature is a non uniform thickening of the leaflet with a fuzzy shape appearance of the thickening. Since the thickening often appears on only a part of the valve it is important that the valves are examined from different directions by making careful M mode scans during continuous recording. Unfortunately we did not have access to a fiber optic recorder allowing a continuous recording at that time. To avoid a false positive diagnosis it is also important to use proper gain setting of the equipment. If the gain settings are too high an unusually thick echo can be obtained from a normal leaflet. Such a false positive diagnosis can be avoided by comparing the echoes obtained with other echoes from the same patient and with the same gain settings. In our patient normal echoes were obtained from the posterior mitral leaflet, aortic leaflets etc.

Possible sources of confusion with respect

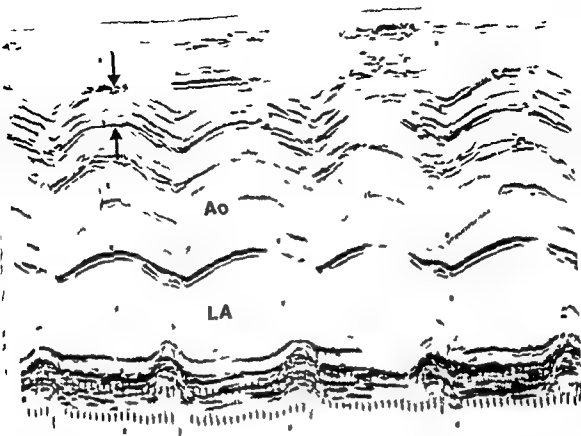


Fig 5 Echocardiogram from patient no 7 obtained with the transducer directed toward the left atrium (LA) the aortic root (Ao) and the right ventricular outflow tract

anterior to the aorta. In the outflow tract an echo producing structure can be seen (arrows) representing the fungal mass.

to these echoes have been discussed by Dillon et al (4) and Martinez et al (14). Calcification of the valves gives a different appearance of the echoes and is unlikely in the pediatric age group. An atrial myxoma can give echoes in the mitral region with some resemblance of the described fuzzy echoes. The myxoma can be detected in the left atrium as well as related to the mitral valve. A differential diagnosis between atrial myxoma and a vegetation on the mitral valve by echocardiography appears possible as described by Dillon et al (4).

In the pediatric age group myxomatous deposits can occur on the mitral or tricuspid leaflets in combination with other congenital heart malformations (9-16). These deposits are often small but it is quite possible that

they can cause echoes similar to those obtained from bacterial vegetations. The echocardiographic findings must therefore be evaluated together with other relevant findings.

Echocardiographic features of tricuspid endocarditis (11, 12) are similar to those reported for mitral endocarditis. In our case 2 a large echo-producing mass was found in the right ventricle during the whole cardiac cycle. Two-dimensional echocardiography was very useful in determining the exact localization of the fungal mass. Right atrial myxomas can give echoes in the same region but then usually only during diastole (5-7). Right ventricular tumors are extremely rare but can give the same echocardiographic appearance as in our case (1, 2, 3, 15). In our case it was not

possible to differentiate between a tumor or another echo-producing structure by echocardiography but the clinical situation made a fungal mass appear very likely.

These case reports underline the value of serial echocardiographic examinations. In the first case the echocardiographic data show the increasing size of the left atrium, the left ventricle and the right ventricle caused by an increasing mitral regurgitation and congestive heart failure. In the second case serial evaluation of tumor size was of value in monitoring the patient and in reaching a decision regarding surgical treatment.

It is quite clear that valvular vegetations can be detected by echocardiography and that two dimensional echocardiography is very helpful in localizing the exact site of the vegetation. What is not known however is how large the vegetation must be before it is detectable echocardiographically or how often vegetations can be detected by this method. Only a systematic study of a large series of patients with endocarditis will answer these questions.

REFERENCES

- Allen H D, Bliden L C, Stone F M, Bessinger F II Jr & Lucas H V Jr. Echocardiographic demonstration of a right ventricular tumor in a neonate. *J Pediatr* 84: 854 1974.
- Chandraratna P A N, San Pedro B, Elkins R C & Grantham N. Echocardiographic angiographic and surgical correlations in right ventricular myxoma simulating valvular pulmonic stenosis. *Circulation* 55: 619 1977.
- De Manry A N, Vismara L A, Miller R R, Neumann A & Mason D T. Unusual echocardiographic manifestations of right and left heart myxomas. *Am J Med* 59: 713 1975.
- Dillon J C, Feigenbaum H, Konecke L L, Davis R H & Chang S. Echocardiographic manifestations of valvular vegetations. *Am Heart J* 86: 698 1973.
- Farooki Z Q, Green E W & Arciniegas E. Echocardiographic pattern of right atrial tumor. *Br Heart J* 38: 580 1976.
- Gottlieb S, Khuddus S A, Bolooki H & Mueburg R J. Echocardiographic diagnosis of endocarditis. *Circulation* 50: Suppl III: 76 1974.
- Harbold N B & Gau G T. Echocardiographic diagnosis of right atrial myxoma. *Mayo Clin Proc* 48: 284 1973.
- Hirschfeld D & Schiffer N B. Echocardiographic localization of aortic valve vegetations. *Circulation* 50: Suppl III: 143 1974.
- Hyams V J & Mannon W C. Incomplete differentiation of the cardiac valves. *Am Heart J* 76: 1: 1963.
- Keith J D, Rowe R D & Vlad P. *Heart disease in infancy and childhood*. The Macmillan Co. N York 1967. 2nd ed. p. 953.
- Kislo J, von Ramm O T, Haney M, Jones I, Jak S S & Behar V S. Echocardiographic evaluation of tricuspid valve endocarditis. *Am J Cardiol* 39: 502 1976.
- Lee C C, Ganguly S N, Magnisalis K & Rot E. Detection of tricuspid valve vegetations by echocardiography. *Chest* 66: 432 1974.
- Lundstrom N R. Clinical applications of echocardiography in infants and children. *Acta Paediatr Scand* Suppl 243: 1974.
- Martinez E C, Burch G E & Giles T D. Echocardiographic diagnosis of vegetative aortic bacterial endocarditis. *Am J Cardiol* 34: 845 1974.
- Nanda N C, Barold S S, Gramiak R C, L S & Heinle R A. Echocardiographic features of right ventricular outflow tumor prolapsing into pulmonary artery. *Am J Cardiol* 40: 272 1977.
- Persson B, Johansson B W, Lundstrom N, Mortenson W & Rausing A. Cor triatriatum. *Acta Paediatr Scand* 61: 477 1972.
- Yoshikawa J, Tanaka K, Owaki T & Kato C. Cord like aortic valve vegetation in bacterial endocarditis. *Circulation* 53: 911 1976.

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RENAL SODIUM EXCRETORY CAPACITY IN INFANTS UNDER DIFFERENT DIETARY CONDITIONS

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ABSTRACT Aperia A, Broberger O, Herin P, Thodenius K and Zetterstrom R (Department of Paediatrics, Karolinska Institutet, St Goran's Hospital, Stockholm, Sweden). Renal sodium excretory capacity in infants under different dietary conditions. *Acta Paediatr Scand* 68:351, 1979. — An evaluation of dietary effects on sodium (Na) homeostasis was performed in 28 healthy infants 7–13 weeks of age. Each infant received during one week an ordinary formula where either the Na and/or the protein content was increased twice. The high Na diets induced a significant elevation of the natriuretic response to an oral Na load. The response was most pronounced in those infants receiving a high Na as well as a high protein diet. The diet that was only high in protein resulted in an increased osmotic load to the kidneys but did not affect the Na excretion. The maturation of renal Na excretion thus seems to be accelerated by a high Na intake and further potentiated by a high protein intake. The Na excretory capacity was even after the period of a high Na diet well above the level of Na then given.

KEY WORDS Infants, sodium excretion, renal function, sodium intake.

In infancy tolerance to sodium (Na) loading as well as to Na depletion is reduced (6, 7). Until recently the interest has been focused on the hazards of a positive Na balance. Since there is experimental evidence of an association between a positive Na balance during development and hypertension in adult life (9, 11) it has been recommended that the daily Na intake should be kept low during the first year of life (1).

The recommended daily Na intake is, however, only an empirical value. Most information on renal handling of Na has been obtained from studies in newborn pre-term (3, 5) and full-term infants (2, 5). The Na excretory capacity in infants older than one month of age has only been studied under standardized dietary conditions with a recommended i.e. low Na intake (4). Under such conditions the capacity to excrete Na will increase linearly during the first year of life.

This study was undertaken to determine whether the tolerance to Na can be influenced by the daily Na intake during the first four months of infancy, i.e. can two to four months old infants tolerate a higher Na intake than is generally recommended if it is given for a longer period of time.

MATERIAL AND METHODS

Twenty-eight healthy infants, born at term and ranging in age from 7 to 13 weeks, were studied. Mothers who had weaned their infants for non-medical reasons were asked in the Well Baby Clinics to participate. For at least four weeks prior to the study all the infants were exclusively fed an ordinary infant formula. Following control determination of the Na excretory capacity, extra Na and/or protein was added to the formula (Baby Semp 1 Semper) for one week. The Na and protein contents for the three different diets are listed in Table 1. In diets where

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Table 1 Sodium (Na) protein and caloric content of the three diets and of an ordinary infant formula

	Na (mmol/l)	Protein (g/l)	Calories (kcal/l)
High Na diet	16	15	675
High Na + protein diet	16	33	670
High protein diet	7	33	680
Ordinary infant formula	7	15	675

Na and/or protein was added the content of each constituent was about twice that in ordinary formulae. In no case did the Na and/or protein enriched diets result in a change of blood pressure, gastrointestinal disturbances or irritability. The Na excretory capacity was determined again at the end of the diet period. Eight infants received the high salt diet, eleven infants received the high salt and high protein diet and nine infants received the high protein diet.

This study has been approved by the Ethical Committee of the Karolinska Institute.

Protocol for the study of the Na excretory capacity

During the study the infants were well hydrated by a diluted ordinary formula given via a stomach tube. The diluted formula was administered in an amount corresponding to 2% of body weight during the first hour and thereafter in an amount corresponding to 0.5% of body weight every half hour. About 90 min after receiving the formula a stable diuresis was attained. A single dose of NaCl (2 mmol Na/kg) was then added to the formula. Urine was obtained by spontaneous voiding. The study was continued during a period of five to six hours after the single dose of NaCl was given. Capillary blood was taken in the middle of the total urine sampling period.

The Na concentration in urine and blood was analyzed using a flame photometer (Eppendorf). Osmolality in urine and blood was determined cryoscopically in a Knauer osmometer. Serum total protein concentration was determined with a refractometric method and serum urea with

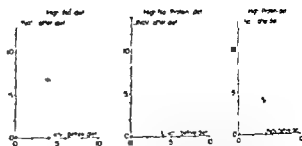


Fig. 1 Dietary effects on sodium excretion (U/V) in the individual infants studied.

a spectrophotometric method. Erythrocyte volume fraction (hematocrit) was estimated by centrifuging the blood in heparinized glass capillaries at 10000 rpm for 5 min. Aldosterone excretion before and towards the end of the diet was determined with a radioimmunoassay method (15) in four infants who received high sodium diet only. Paired *t* test has been used in the statistical evaluation. Each infant has been its own control.

RESULTS

Some general effects of the three different diets are summarized in Table 2. The daily weight gain was higher in the infants given a high Na and high protein diet but the difference from the other two groups was not significant. The hematocrit fell slightly but not significantly in the groups that received a high protein diet. The serum protein concentration did not change with the different diets but the serum urea concentration increased significantly in the infants who were given high protein diets. The serum Na concentration was not affected by any of the three diets.

Table 2 General effects of high sodium (Na) and high protein diet. Values are mean \pm standard deviation. NS = not significant.

	Daily weight gain (g)	Hematocrit (B-EVF) (%)		S Protein (g/l)	
		Before diet	After diet	Before diet	After diet
High Na diet (n=8)	76.0 \pm 10.8	34.1 \pm 2.41	34.7 \pm 1.98	62.0 \pm 3.16	60.0 \pm 7.87
<i>p</i> value		NS		NS	
High Na + protein diet (n=11)	77.7 \pm 10.1	37.1 \pm 4.62	35.6 \pm 1.67	61.2 \pm 5.12	62.3 \pm 5.36
<i>p</i> value		NS		NS	
High protein diet (n=9)	30.1 \pm 10.1	42.4 \pm 11.9	40.1 \pm 9.01	65.1 \pm 4.51	64.8 \pm 3.19
<i>p</i> value		NS		NS	

Table 3 Renal response to an oral sodium (Na) load during different dietary conditions

Values are mean \pm standard deviation N S = not significant

	[Na (mmol/l 73 m /hour)		Urinary flow (l/1 73 m /hour)		C_{Na} (l/1 73 m /hour)	
	Before diet	After diet	Before diet	After diet	Before diet	After diet
High Na diet ($n=8$)	3.35 \pm 1.86	4.90 \pm 2.16	0.7 \pm 0.05	0.6 \pm 0.05	0.07 \pm 0.04	0.09 \pm 0.07
<i>p</i> value		<0.05		<0.05		N S
High Na + protein diet ($n=11$)	4.10 \pm 2.18	8.06 \pm 2.77	0.6 \pm 0.04	0.27 \pm 0.08	0.09 \pm 0.06	0.19 \pm 0.05
<i>p</i> value		<0.001		N S		<0.01
High protein diet ($n=9$)	4.69 \pm 3.38	4.85 \pm 2.76	0.0 \pm 0.06	0.71 \pm 0.08	0.08 \pm 0.03	0.17 \pm 0.02
<i>p</i> value		N S		N S		<0.05

The capacity to excrete an oral Na load increased significantly in both groups receiving a high Na diet but in the group receiving only a high protein diet the capacity to excrete Na was unchanged after the diet. The increase in Na excretory capacity was more pronounced following a high Na and high protein diet than following a high Na diet only (Fig. 1 Table 3). The increase following a high Na and high protein diet was 2 fold and following a high Na diet 1.5 fold. In the high protein group the relationship between Na excretion before and after the diet was 1.0. Urinary flow (Table 3) increased slightly in the group receiving only a high Na diet but not in the other two groups. As expected from the increased serum urea values the osmolar clearance increased significantly in the two groups receiving high protein diets. It was unchanged in the group receiving only a high Na diet. Aldosterone ex-

cretion before and towards the end of the diet was determined in four infants who only received a high sodium diet. The mean difference before and after the diet was not significant.

DISCUSSION

The infant kidney is usually unable to rapidly excrete a given Na load. When however the daily Na intake is increased for a week as in this study the capacity to excrete Na increases. The results accord with the findings in previous experimental studies on puppies in which the increase in Na excretory capacity was attributed to both an increased glomerular filtration rate and to tubular rejection of Na (16).

In the present study the capacity to excrete Na was further augmented when a high protein and high Na diet was administered. It is thought that a high protein diet can influence the function of the infant kidney by 1) enhancement of growth and 2) increase in osmotic load secondary to the resulting increase in serum urea concentration (10, 12). A high protein diet caused a rise in serum urea concentration and hence in the osmolar clearance but did not in itself affect Na excretion. This indicates that the enhancement of Na excretory capacity observed following a high Na and high protein diet was not due to transitory changes in the osmotic load. It is therefore suggested that the high salt diet induces func-

Urea (mmol/l)		S-Na (mmol/l)	
Before diet	After diet	Before diet	After diet
4.0 \pm 0.6	5.40 \pm 2.38	140.0 \pm 3.34	141.0 \pm 2.77
	N S		N S
7.0 \pm 0.71	8.91 \pm 1.83	140.9 \pm 0.81	140.5 \pm 3.88
	<0.001		N S
0.11 \pm 0.18	0.64 \pm 0.03	138.0 \pm 3.00	138.8 \pm 2.64
	<0.001		N S

Table 1 Sodium (Na) protein and caloric content of the three diets and of an ordinary infant formula

	Na (mmol/l)	Protein (g/l)	Calories (kcal/l)
High Na diet	16	15	675
High Na+protein diet	16	33	670
High protein diet	7	33	680
Ordinary infant formula	7	15	675

Na and/or protein was added the content of each constituent was about twice that in ordinary formula. In no case did the Na and/or protein enriched diets result in a change of blood pressure, gastrointestinal disturbances or irritability. The Na excretory capacity was determined again at the end of the diet period. Eight infants received the high salt diet, eleven infants received the high salt and high protein diet and nine infants received the high protein diet.

This study has been approved by the Ethical Committee of the Karolinska Institute.

Protocol for the study of the Na excretory capacity

During the study the infants were well hydrated by a diluted ordinary formula given via a stomach tube. The diluted formula was administered in an amount corresponding to 2% of body weight during the first hour and thereafter in an amount corresponding to 0.5% of body weight every half hour. About 90 min after receiving the formula a stable diuresis was attained. A single dose of NaCl (2 mmol Na/kg) was then added to the formula. Urine was obtained by spontaneous voiding. The study was continued during a period of five to six hours after the single dose of NaCl was given. Capillary blood was taken in the middle of the total urine sampling period.

The Na concentration in urine and blood was analyzed using a flame photometer (Eppendorf). Osmolality in urine and blood was determined cryoscopically in a Knauer osmometer. Serum total protein concentration was determined with a refractometric method and serum urea with

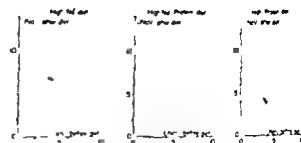


Fig. 1 Dietary effects on sodium excretion ($U_{Na} V$) in the individual infants studied.

a spectrophotometric method. Erythrocyte volume fraction (hematocrit) was estimated by centrifuging the blood in heparinized glass capillaries at 10000 rpm for 5 min. Aldosterone excretion before and towards the end of the diet was determined with a radioimmunoassay method (15) in four infants who received high sodium diet only. Paired *t* test have been used in the statistical evaluation. Each infant has been its own control.

RESULTS

Some general effects of the three different diets are summarized in Table 2. The daily weight gain was higher in the infants given a high Na and high protein diet but the difference from the other two groups was not significant. The hematocrit fell slightly but not significantly in the groups that received a high protein diet. The serum protein concentration did not change with the different diets but the serum urea concentration increased significantly in the infants who were given high protein diets. The serum Na concentration was not affected by any of the three diets.

Table 2 General effects of high sodium (Na) and high protein diet

Values are mean \pm standard deviation. NS = not significant

	Daily weight gain (g)	Hematocrit (B-H V) (%)		% Protein (g/l)	
		Before diet	After diet	Before diet	After diet
High Na diet ($n=8$)	26.0 \pm 10.8	34.1 \pm 2.41	34.7 \pm 1.98	62.0 \pm 3.16	60.0 \pm 7.87
<i>p</i> value		NS		NS	
High Na+protein diet ($n=11$)	33.7 \pm 10.1	37.1 \pm 4.67	35.6 \pm 1.67	61.2 \pm 5.12	67.3 \pm 5.36
<i>p</i> value		NS		NS	
High protein diet ($n=9$)	30.1 \pm 10.1	42.4 \pm 11.9	40.1 \pm 9.01	65.1 \pm 4.51	64.8 \pm 1.19
<i>p</i> value		NS		NS	

- 4 Aperia A, Broberger O, Thodenius K & Zetterstrom R. Development of renal control of salt and fluid homeostasis during the first year of life. *Acta Paediatr Scand* 64: 393 1975
- 5 Aperia A, Broberger O, Thodenius K & Zetterstrom R. Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy. *Acta Paediatr Scand* 64: 775 1975
- 6 Aperia A, Broberger O, Thodenius K & Zetterstrom R. Control of sodium homeostasis in newborn infants. In *Intensive care in the newborn. Symposium Stockholm*. Middelbart, Denmark, August 25-30 1974. Interscience Publ (John Wiley & Son)
- 7 Aperia A, Broberger O, Broberger U, Larsson L, Thodenius K & Zetterstrom R. Sodium and fluid homeostasis in normal and diseased newborn. In *Symposium on intensive care in the newborn*. Durham, New Hampshire, USA, August 8-September 1977.
- 8 Aperia A, Ehnder G, Henn P & Kallskog O. Renal hemodynamics and filtration rate in growing rats. Effect of isotonic volume expansion. *27th International Congress of Physiology*, Paris 1977.
- 9 Bianchi M, Baer M, G Fox U & Guidi E. The role of the kidney in the rat with genetic hypertension. *Physiol Med J* 53: 193 1977.
- 10 Calcagno P L & Lowe C U. Substrate induced renal tubular maturation. *J Pediatr* 63: 851 1963.
- 11 Dahl L K. Salt in processed baby foods. *Am J Clin Nutr* 21: 787 1968.
- 12 Edelmann C M Jr & Wolfish N M. Dietary influence on renal maturation in premature infants. *Pediatr Res* 2: 471 1968.
- 13 Horster M & Larsson L. Mechanisms of fluid absorption during proximal tubule development. *Kidney Int* 10: 348 1976.
- 14 Jose P A, Slotkoff L M, Lillienfeld L S, Calcagno P L & Eisner G M. Sensitivity of neonatal renal vasculature to epinephrine. *Am J Physiol* 226: 796 1974.
- 15 Sealey J E, Buhler F R, Laragh J H, Manning E L & Brunner H R. Aldosterone excretion. *Circ Res* 31: 367 1977.
- 16 Steichen J J & Kleinman L I. Influence of dietary sodium intake on renal maturation in unanesthetized canine puppies. *Proc Soc Exp Biol Med* 148: 748 1975.

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Table 4 The maximal sodium (Na) excretory capacity in relation to Na intake during different dietary conditions

Values are mean \pm standard deviation. N.S. = not significant

	Na excretion following load (mmol/kg/6 hours)		Na intake (mmol/kg/24 hours)	
	Before diet	After diet	Before diet	During diet
High Na diet (n=8)	0.64 \pm 0.31	0.89 \pm 0.38	1.19 \pm 0.14	2.60 \pm 0.41
High Na protein diet (n=11)	0.74 \pm 0.32	1.43 \pm 0.46	1.04 \pm 0.21	2.28 \pm 0.40
High protein diet (n=9)	0.84 \pm 0.46	0.83 \pm 0.45	1.19 \pm 0.10	1.13 \pm 0.10

tional changes that will increase Na excretion and that those changes will be more pronounced if renal growth is facilitated at the same time. If one considers the possibility that growth will potentiate the enhanced Na excretory capacity following the diet, there are at least two possible explanations for this effect.

1. Na excretion is increased due to a rise in filtered Na. Glomerular filtration rate is low in the infant kidney due in part to high renal vascular resistance (8). High salt diet will result in vasodilatation and the increase in renal blood flow and glomerular filtration rate will be further augmented by a growth conditioned increase in vascular volume.

2. Na excretion is increased due to reduced fractional Na reabsorption across the paracellular pathway. The water permeability of the paracellular pathway is high in the infant kidney due to leakiness of the paracellular junction (13). The hemodynamics of the infant kidney will result in hydrostatic pressure gradients that favour a passive Na reabsorption across the junction (8). High Na diet might then change the pressure gradients across the paracellular junctions and high protein diet might alter the structure of the paracellular junctions resulting in a decreased water permeability.

It has been shown in a previous study (2) that the Na excreted following the load of 2 mmol/kg most likely approximates the maximal Na excretory capacity in infants. In infants who received a high Na diet the Na ex-

cretion after the salt load averaged 0.88 mmol/kg/6 hours or 3.52 mmol/kg/24 hours (Table 4). For several weeks prior to the study all the infants were given a regular formula providing a daily Na intake of about 1.2 mmol/kg while on the high Na diet they received a daily Na intake of about 2.6 mmol/kg. Hence the maximal Na excretory rate after the diet considerably exceeded the intake during the high Na diet. Thus the high Na diet should not result in an accumulation of Na with or without fluid. The fact that the diet did not affect the serum Na concentration and hematocrit also indicates that this was not the case. The present results thus suggest that the increased daily Na intake for a period of at least a week will contribute to the development of the Na excretory capacity. It might therefore be an advantage to increase the daily Na intake stepwise during the first months of life provided that infants are healthy, show no signs of renal disease and have a negative family history of hypertension.

REFERENCES

1. American Academy of Pediatrics Committee on Nutrition. Salt intake and eating patterns of infants and children in relation to blood pressure. *Pediatrics* 53: 115, 1974.
2. Aperia A, Broberger O, Thodenius K, & Zetterstrom R. Renal response to an oral sodium load in newborn full term infants. *Acta Paediatr Scand* 61: 670, 1972.
3. Aperia A, Broberger O, Thodenius K, & Zetterstrom R. Developmental study of the renal response to an oral salt load in preterm infants. *Acta Paediatr Scand* 63: 517, 1974.

- 4 Aperia A, Broberger O, Thodenius W & Zetterstrom R. Development of renal control of salt and fluid homeostasis during the first year of life. *Acta Paediatr Scand* 64: 393 1975
- 5 Aperia A, Broberger O, Thodenius K & Zetterstrom R. Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy. *Acta Paediatr Scand* 64: 75 1975
- 6 Aperia A, Broberger O, Thodenius K & Zetterstrom R. Control of sodium homeostasis in newborn infants. In *Intensive care in the newborn Symposium* Starb SLO, Middelfart, Denmark, August 25-30 1975. Interscience Publ. (John Wiley & Son)
- 7 Aperia A, Broberger O, Broberger U, Larsson L, Thodenius K & Zetterstrom R. Sodium and fluid homeostasis in normal and diseased newborn. In *Symposium on intensive care in the newborn*. Durham, New Hampshire, USA, August 8-September 1977
- 8 Aperia A, Elinder G, Henn P & Kallskog O. Renal hemodynamics and filtration rate in growing rats. Effect of isotonic volume expansion. *7th International Congress of Physiology*, Paris 1977
- 9 Bianchi G, Baer P G, Fox U & Gudis E. The role of the kidney in the rat with genetic hypertension. *Postgrad Med J* 53: 13 1977
- 10 Calcagno P L & Lowe C U. Substrate induced renal tubular maturation. *J Pediatr* 63: 851 1963
- 11 Dahl L K. Salt in processed baby foods. *Am J Clin Nutr* 21: 787 1968
- 12 Edelmann C M Jr & Wolfish N M. Dietary influence on renal maturation in premature infants. *Pediatr Res* 2: 41 1968
- 13 Horster M & Larsson L. Mechanisms of fluid absorption during proximal tubule development. *Kidney Int* 10: 348 1976
- 14 José P A, Slotkoff L M, Lillienfeld L S, Calcagno P L & Eisner G M. Sensitivity of neonatal renal vasculature to epinephrine. *Am J Physiol* 226: 796 1974
- 15 Sealey J E, Bühler F R, Laragh J H, Manning E L & Brunner H R. Aldosterone excretion. *Circ Res* 31: 367 1972
- 16 Steichen J J & Kleinman L I. Influence of dietary sodium intake on renal maturation in unanesthetized canine puppies. *Proc Soc Exp Biol Med* 148: 748 1975

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SERIAL MEASUREMENTS OF THORACIC IMPEDANCE AND CARDIAC OUTPUT IN HEALTHY NEONATES AFTER NORMAL DELIVERY AND CAESAREAN SECTION

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ABSTRACT Freyschuss U Noack G and Zetterstrom R (Departments of Clinical Physiology and of Pediatrics Karolinska Institute at Karolinska Hospital and St Goran's Hospital Stockholm Sweden) Serial measurements of thoracic impedance and cardiac output in healthy neonates after normal delivery and caesarean section. *Acta Paediatr Scand* 68 357 1979.—Thoracic electrical impedance measurements were serially performed during the 1st 2nd 8th and up to the 32nd hour of life in two groups (V and S) of healthy infants. In group V all 24 infants were delivered vaginally. In group S all 24 infants were delivered by caesarean section for obstetrical reasons. Basal thoracic impedance (Z) heart rate (HR) stroke volume (SV) and cardiac output (Q) were determined on each examination. In group V Z increased from 31.9 to 34.0 ohm between 2 hours and the last recording between 8 and 32 hours. SV decreased from 4.1 to 3.4 ml between 2 and 4 hours and was accompanied by a decrease of Q from 560 to 450 ml/min. Heart rate slowed from 129 to 115 beats/min between 2 hours and the last recording at ≥ 8 hours. In group S Z increased from 32.2 to 35.9 ohm between 2 and 8 hours. Mean SV increased from 3.6 to 4.4 ml between 8 and 32 hours and heart rate slowed from 131 to 113 beats/min between 1 and 8 hours. No significant differences were observed between the groups. The accuracy of the impedance—SV and Q data cannot be validated. For the most part they compare favourably with values previously obtained by soluble gas methods. Serial changes may reflect not only decreasing shunts and/or increasing aeration but also changes in total fluid volume of the lungs intra- or extravascular. The precision of the measurements is good since reproducibility of single SV and Q determinations is higher than with standard dilution techniques. The data obtained may serve as baseline values for comparison with data in infants of the same age with various anomalies.

KEY WORDS Cardiac output newborns section thoracic impedance

Monitoring of cardiovascular function in the neonatal period has been impeded by the lack of atraumatic non invasive techniques. The recent development and increased clinical application of transthoracic electrical impedance measurements (for review see Geddes & Baker (11)) provide a simple but sensitive method for study of changes in intrathoracic air fluid ratios (3 9 19 24 29 30 34) as for example in pulmonary edema and pleural effusion (19 30 34). In infants the technique has been used in apnoea monitoring (8 12 26 27 33). Since the transthoracic impedance system was developed for measurement of cardiac stroke volume (15 16 28) much interest has been

displayed in the use of this non invasive method for cardiac output determination. Thus it has been used during anaesthesia surgery and in patients with myocardial infarction (10 13 18 30). In children with congenital heart disease cardiac output data obtained by impedance cardiography and by dye dilution or Fick principle (17) were found to be comparable in subjects without intracardiac shunts or valvular insufficiencies. In patients with left to right shunts the impedance cardiac output values showed a good correlation with pulmonary blood flow (17).

In the neonatal period measurement of the intrathoracic air fluid ratio and stroke vol

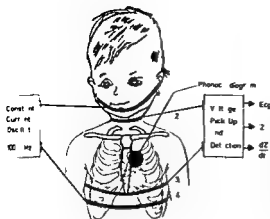


Fig 1 Recording procedure. Explanation is given in text.

Three output signals were recorded on a multi-channel graphic recorder (Mingograph Siemens Elema Sweden) an ECG (taken with electrodes 1 and 4) dZ/dt and phonocardiogram. As the baseline shifted with respiration only tracings at the zero line were used for calculations. i.e. the same procedure employed in recordings of apnoea.

In each subject the average of five beats was used to calculate one stroke volume. Cardiac output was determined by multiplying the stroke volume by heart rate which was obtained from the ECG. Each measurement represents the mean value of two or three determinations.

A complete series of observations i.e. at 1 2 4 8 16 4 and 32 hours of life was usually not obtained as no measurements were made in the evening or at night.

The level of significance between paired observations or group means is indicated as follows: $p < 0.001$ $p < 0.01$ $p < 0.05$ not significant = N.S.

RESULTS

Basal impedance (Z), stroke volume, heart rate and cardiac output on each examination were analyzed and compared both within and between the two groups. Mean values \pm S.D. are given in Fig. 2.

In group V paired observations indicated an average increase of Z from 31.9 to 34.0 ohm ($p < 0.05$) between the measurements at 2 hours and the time for the last recording at 8, 24 or 32 hours. Paired differences between other intervals were not significant. The estimated stroke volume decreased from a mean of 4.1 to 3.4 ml ($p < 0.01$) between 2 and 4 hours and the change was associated with a decrease of cardiac output from a mean of 560 ml/min

to 450 ml/min ($p < 0.05$). Both variables increased between 4 and 32 hours to 4.9 ml ($p < 0.01$) and 557 ml/min ($p < 0.05$) respectively. Within other time intervals neither of the two variables changed significantly. The mean heart rate slowed from 129 to 115 bpm ($p < 0.01$) between 2 hours and the hour of the last recording at 8 hours or later.

In group S serial observations demonstrated an increase of Z from a mean of 32.2 to 35.9 ohm ($p < 0.05$) between 2 and 8 hours; no significant changes were noted within other intervals.

The mean value of stroke volume increased from 3.6 to 4.4 ml ($p < 0.05$) between 8 and 32 hours. Cardiac output increased within the same interval from an average of 406 ml/min to 506 ml/min ($p < 0.01$). Other changes in the two variables were not significant. The mean heart rate slowed from 131 to 113 bpm ($p < 0.01$) between 1 and 8 hours and did not significantly change thereafter.

On comparing the mean values in groups V and S at comparable hours the only differences demonstrated were a somewhat greater stroke volume in group S at 4 hours ($p < 0.05$) and a higher Z at 8 hours within the same group ($p < 0.01$).

The reproducibility of a single Z_0 determination using the formula $c = SD_{diff} / 100 / \sqrt{2} \times$

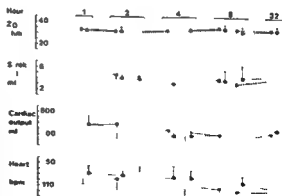


Fig. 2 Serial changes in basal impedance (Z), stroke volume, cardiac output and heart rate in group V (●) (vaginal delivery) and group S (■) (caesarean section). Paired mean differences \pm S.D. are given.

Table 1 Vital characteristics of healthy newborns delivered normally (V) and by caesarean section (S)

Mean values \pm S D and significance (sig) of mean value differences are given

	V	Sig	S
Number	24		24
Weight kg	3 520 \pm 454	NS	3 327 \pm 534
Length cm	51 \pm 1.8	*	49 \pm 1.7
Apgar score			
1 min			9.0 \pm 0.6
5 min			10.0 \pm 0.0
Distance between electrodes 2 and 3 cm	7.1 \pm 0.6	NS	7.3 \pm 0.6
Circumference electrode 3 cm	34.4 \pm 1.5	NS	33.4 \pm 2.2
Mother's age yr	27.9 \pm 4.7	NS	29.6 \pm 4.9

ume-cardiac output should be of clinical value in the assessment of various conditions associated with respiratory distress. No data on such stroke volume measurements in healthy term infants have, however, been found in a search of the literature. In a recent report Godfrey and co-workers (6) presented cardiac output measurements obtained with impedance cardiography in 32 pre-term infants of 2-81 days of age. The data are of limited value for reference purposes, however, because of the wide variations in gestational and actual age. Serial measurements are particularly needed for the first day of life when shunts of varying direction may be present. For this reason the aim of this study was to obtain serial observations on the intrathoracic air/fluid ratio as reflected by basal impedance and on the cardiac output during the first day of life in healthy newborns. A comparison will also be made with a series of healthy infants delivered by caesarean section with a view to observing changes in the relation pulmonary air/fluid during adaptation to extruterine life. Finally it is hoped that the data acquired shall provide baseline values for comparison with those obtained from a current investigation of subjects with various conditions of the same age. The value of monitoring intrathoracic impedance in infants with respiratory diffi-

culty for in early diagnosis of pneumothorax has already been reported (22).

MATERIAL

Group V (Table 1) consisted of 24 healthy infants (7 girls, 17 boys) who were born at term to healthy mothers after spontaneous uncomplicated vaginal deliveries. Group S (Table 1) consisted of 24 healthy infants (15 girls, 9 boys) who were delivered by caesarean section at an average of 39 weeks of gestational age. Only healthy mothers were included in the study and the indications for section were obstetric: i.e. abnormal pelvis ($n=14$), placenta praevia ($n=1$), breech and crown presentations ($n=2$), movable foetal head ($n=1$), elderly primiparae or prolonged delivery ($n=4$), uterine fistula ($n=1$) and suspected placental insufficiency ($n=1$). In both groups the infants were in good condition and the perinatal course was uneventful. The umbilical cord was clamped within about one minute at the vaginal deliveries and usually within 30-45 sec in caesarean sections. In group S capillary hematocrits were obtained in 12 of the subjects within two hours after birth. Ordinary values were measured (mean $59\pm 8.8\%$).

METHOD

Thoracic impedance data were obtained using four aluminumized Mylar tape electrodes (3 M Co. Minnesota) attached around the patient's chest (see Fig. 1) and an impedance cardiograph (IFM Minnesota Model 104A). A constant sinusoidal alternating current of 4 mA at a frequency of 100 kHz was applied to the outer electrodes 1 and 4, and from the two inner electrodes 2 and 3 the standing impedance (i.e. basal impedance (Z_0)) was read on a digital display. Both potential and current electrodes are internally connected to isolation transformers so that the maximal possible earth leakage current to the patient is limited to 40 μ A. A crystal microphone was placed over the second left intercostal space in order to record a phonocardiogram thus facilitating proper timing of the 2nd heart sound. The electrodes, which are radiolucent to X-ray, were kept in place during all of the recordings. Stroke volume was calculated using a modification of Kubicek and co-workers' method (15) with the formula

$$\text{Stroke volume} = P(L/Z)^2 T dZ/dt$$

where P =hematocrit-dependent resistivity of blood (ohm cm) (15), L =distance (cm) between electrodes 2 and 3, Z_0 =basal impedance (ohm), T =ventricular ejection time (sec) and dZ/dt (ohm/sec)=the first derivative of the diminution of Z_0 occurring during the rapid ejection phase of the cardiac cycle. According to this formula the region between electrodes 2 and 3 is regarded as a cylindrical homogenous conductor in which there is a proportional change in basal impedance with the ejection of each stroke volume. A value of 257 ohm cm was used for resistivity of blood since it corresponds to a hematocrit of 60% i.e. the average hematocrit in infants (23). This value was not significantly different from that measured in group S.

tions in hemodynamic data with time after birth

In the present study the accuracy of the stroke volume-cardiac output data cannot be validated. In comparison to the few available cardiac output determinations in healthy infants (1) the values obtained by the impedance technique are about 30% lower but of higher reproducibility. Our results (165 to 159 ml/min body weight) obtained from the 1st to the 32nd hour compare favourably with estimations of Qpc eff (4, 5) and cardiac output (31) in healthy infants of the same gestational age and during the first day of life. The values are lower than those obtained by Costeloe et al. in pre-term infants (mean 205 ml/kg BW) which may be related both to the different resistivity factor used and to the significantly lower hematocrit values in the latter study where the majority were less than 50%.

Serial cardiac output measurements such as those reported here have not been found in the literature where repeated observations usually represent grouped mean values obtained at different ages during the first days of life (4, 7) the only consistent changes with time in both groups that were found in this study were a slowing of the heart rate and an increase in the air fluid ratio (Z). Both variables may indicate a decreasing left to right shunt and/or in the case of Z an increasing aeration of the lungs. In conclusion the chief value of impedance cardiography as an investigative method in neonates is probably its ability to monitor and detect deviations from the normal sequence of cardiopulmonary adaptation.

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REFERENCES

- 1 Artz R A, William O, Wallgren G, Hanson J, Gesner I H & Lind J. Quantitative studies of the human neonatal circulation. *Acta Paediatr Scand* S ppl 179 196 pp 3-4
- Baker L E, Judy W & Geddes L E. Langley

- F M & Hill D W. The measurement of cardiac output by means of electrical impedance. *Cardiovasc Res Cent Bull* 9 135 1971
- 3 Berman I H, Scheetz W L, Jenkins E B & Hufnagel H V. Transthoracic electrical impedance as a guide to intravascular overload. *Arch Surg* 107 61 1971
- 4 Brady J P & Rigatto H. Pulmonary capillary flow in the newborn infant. A new method using the plethysmograph and nitrous oxide. *Pediatrics* 43 707 1971
- 5 Chu J, Clements J A, Cotton E K, Klaus M H, Sweet A Y & Tooley W H. Neonatal pulmonary ischemia. *Pediatrics* 40 709 1967
- 6 Costeloe K, Stocks J, Godfrey S, Mohapatra S N & Hill H W. Cardiac output in the neonatal period using impedance cardiography. *Pediatr Res* 11 1171 1977
- 7 Cotton E K, Cogswell J J & Cropp G J A. Measurements of effective pulmonary blood flow in the normal newborn human infant. *Pediatrics* 47 50 1971
- 8 Daily W J R, Klaus M & Meyer H B P. Apnea in premature infants: monitoring incidence, heart rate changes, and an effect of environmental temperature. *Pediatrics* 43 510 1969
- 9 Dennison J C & Baker L E. Measurement of pleural effusion by electrical impedance. *J Appl Physiol* 38 851 1975
- 10 Gabriel S, Atterhog J H, Oro L & Ekelund L G. Measurement of cardiac output by impedance cardiography in patients with myocardial infarction. *Scand J Clin Lab Invest* 36 29 1976
- 11 Geddes L A & Baker L E. *Principles of applied biomedical instrumentation*. Wiley, New York 1968
- 12 Hamilton L H & Burns W T. Impedance measuring ventilation monitor for infants. *Ann Biomed Eng* 1 3 4 1973
- 13 Hill D W & Lowe H J. The use of the electrical impedance technique for the monitoring of cardiac output and limb bloodflow during anaesthesia. *Med Biol Eng* 11 534 1973
- 14 Hill D W & Thompson F H. The effect of haematocrit on the resistivity of human blood at 37°C and 100 kHz. *Med Biol Eng* 13 18 1975
- 15 Kubicek W G, Karnegs J N, Patterson R P, Witsoe D A & Mattison R H. Development and evaluation of an impedance cardiac output system. *Aerospace Med* 37 108 1966
- 16 Kubicek W G, Patterson R P & Witsoe D A. Impedance cardiography as a noninvasive method to monitor cardiac function and other parameters of the cardiovascular system. International conference on bioelectrical impedance. *Ann NY Acad Sci* 170 7-4 1970
- 17 Lababidi Z, Ehrhke D A, Durkin R E, Leaverton H E & Lauer R M. Evaluation of impedance cardiac output in children. *Pediatrics* 47 870 1971
- 18 Lenz H J, Thomas T A & Wilkins D G. Cardiovascular changes during laparoscopy. Studies of stroke volume and cardiac output using impedance cardiography. *Anaesthesia* 31 4 1976

averaged 2.8% ($p < 0.05$). Corresponding values for stroke volume and cardiac output were 9.9 ($p < 0.05$) and 10.3 ($p < 0.05$)% respectively.

DISCUSSION

Non invasive measurements of cardiac output in infants have hitherto relied upon methods determining the rate of uptake of an inert soluble gas, e.g. freon (5) or nitrous oxide (N₂O) (4, 6, 7, 25). As blood bypassing ventilated portions of the lung cannot be measured the methods are unreliable in patients with shunts or with uneven ventilation in relation to perfusion. The flow measured is therefore called effective pulmonary blood flow ($Q_{pc\ eff}$). The distinction between $Q_{pc\ eff}$ and true pulmonary blood flow should be kept in mind.

Impedance cardiography offers an even more atraumatic, rapid and easy technique for the determination of pulmonary blood flow. Experimental and clinical investigations have demonstrated good reproducibility, being usually better than for invasive standard dilution techniques (2, 10, 15). In the presence of left to right shunts impedance cardiac outputs correlate with pulmonary blood flow rather than with systemic blood flow (17).

Recently Godfrey and co-workers compared impedance cardiac output data with $Q_{pc\ eff}$ measured by the N₂O rebreathing technique in 32 premature infants and found significant correlations between the methods. Interest was focused on the effect of the hematocrit dependent electrical resistivity of blood on the calculated impedance stroke volume. With the resistivity factor (20) they used cardiac output determined by the impedance technique was significantly higher than the corresponding values obtained with the rebreathing technique in infants with hematocrits above 35%. Using Kubicek's data however, the two cardiac output methods correlated fairly well. A comparison of cardiac output data in anemic adult patients using the same impedance technique and a radioisotope

technique also revealed a good correlation $r = 0.86$ (14).

From what was said earlier it is obvious that the rebreathing technique is not ideal as a reference method because of its inability to measure shunted blood. Left to right shunts may possibly be detected by recirculation of the tracer gas while neither right to left shunts nor abnormalities of ventilation and perfusion can be recognized. Shunts cannot be differentiated by the impedance technique either since the short circuited flow will be included in the cardiac output.

In the present study the reduction of cardiac output in normally delivered infants between 2 and 4 hours of life may be related to the disappearance of shunting previously demonstrated by other authors with oximetry (21, 32) and dye dilution (1). The rise of the intrathoracic air fluid ratio i.e. Z_0 between 2 hours and the last recording are in accordance with the increase of Z_0 from 2 to 12 hours of life that was observed by Olsson & Victorin (24). Although the absolute values are not comparable because of differences in technique and instrumentation the changes are relevant.

Olsson & Victorin have reported a more rapid rise of the transthoracic impedance in vaginally delivered infants with late clamping of the umbilical cord and hence a larger blood volume than in early clamped children. The observations in group S of a more rapid sequence of Z_0 changes and of a larger average stroke volume at 4 hours after birth than the corresponding mean value in group V can be attributed to a common hemodynamic etiology viz a larger blood volume.

The very few differences between groups V and S mean values (variance being similar) at the same times of measurement are striking. On the other hand in view of the uneventful perinatal course there is no reason to believe that the measured cardiovascular variables should differ greatly from the healthy control material. Thus the difference between the groups should rather be the sequence of varia-

SCREENING OF CORD BLOOD LOW DENSITY LIPOPROTEIN CHOLESTEROL IN THE DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLAEMIA A STUDY OF 2000 INFANTS

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ABSTRACT Boulton T J C, Craig I H and Hill G (Adelaide Children's Hospital, Royal Adelaide Hospital and Departments of Paediatrics and Medicine, University of Adelaide, Adelaide, Australia). Screening of cord blood low-density lipoprotein cholesterol in the diagnosis of familial hypercholesterolaemia. A study of 2000 infants. *Acta Paediatr Scand* 68: 363, 1979.—A prospective follow up study of infants selected by cord blood total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels from 2000 consecutive live births was undertaken to reassess the role of cord blood screening in the diagnosis of familial hypercholesterolaemia (FH). Mean values for serum cholesterol were (mmol/l \pm SD): TC 1.63 ± 0.46 , LDL-C 0.90 ± 0.49 , HDL-C 0.70 ± 0.33 , TG 0.38 ± 0.16 . Seventy three of 117 infants who had had a cord TC and/or LDL-C > 9 th percentile and 373 control group children (cord TC and/or LDL-C < 95 th percentile) were followed up at age 3-12 months. Six of the 117 were hypercholesterolaemic (HC) and one child had an HC parent. Positive detection rate ≥ 0.85 , false positive rate $\geq 3.7\%$. Four control-group children were HC and had an HC parent. False negative rate $\geq 1.1\%$. With the possible exception of detecting FH in a child with a known affected parent, cord blood screening appears to be unreliable for the diagnosis of FH.

KEY WORDS Cord blood screening, cord serum low-density lipoprotein cholesterol, familial hypercholesterolaemia.

An elevated serum cholesterol may be due to familial hypercholesterolaemia (FH) which is one of the commonest inborn errors of metabolism (11). The ability to detect this condition in early life may allow long term prophylactic measures to be taken in one group of people with an increased risk of premature ischaemic heart disease (18). Although the excess risk in FH may be partly independent of the actual level of serum cholesterol (28), early intervention to reduce an elevated serum cholesterol level is becoming therapeutically acceptable (19, 32).

The identification of newborn infants with FH by cord blood lipid analysis has been attempted by several groups of investigators but with conflicting results (2, 10, 15, 16, 17, 20, 27, 31). The proportions of false positive results varied considerably in these studies

and the validity of cord serum LDL-C as a marker for future HC remains unproven.

Tsang (32) suggested that a Prospective follow up of much larger numbers of children selected by cord blood low density lipoprotein cholesterol levels will be required to definitively assess the question of false negative cord blood cholesterol levels.

Thus a prospective follow up study was planned in an attempt to answer three questions.

First, does an elevated cord serum cholesterol level predict future hypercholesterolaemia in infancy and childhood? Second, does LDL-C provide a more sensitive marker for future hypercholesterolaemia than TC? Third, what proportion of children with normal cord serum lipid levels subsequently become hypercholesterolaemic?

- 19 Luepker R V, Michael J H & Warbrasse J R. Transthoracic electrical impedance. Quantitative evaluation of a non invasive measure of thoracic fluid volume. *Am Heart J* 85 83 1973
- 20 Mohapatra S N, Costelloe K L & Hill E W. Blood resistivity and its implications for the calculation of cardiac output by the thoracic electrical impedance technique. *Intensive Care Med* 3 63 1977
- 21 Moss A J, Emmrouhides G & Duffie E H Jr. Closure of the ductus arteriosus in the newborn infant. *Pediatrics* 32 25 1963
- 22 Noack G & Freyschuss U. The early detection of pneumothorax with transthoracic impedance in newborn infants. *Acta Paediatr Scand* 66 677 1977
- 23 Oh W & Lind J. Venous and capillary hematocrit in newborn infants and placental transfusion. *Acta Paediatr Scand* 55 38 1966
- 24 Olsson T & Victorin I. Transthoracic impedance with special reference to newborn infants and the ratio air to fluid in the lungs. *Acta Paediatr Scand Suppl* 207 1970
- 25 Orme R I, Featherby E A, Rigatto H, Cervantes F J & Brady J P. Effective pulmonary blood flow in preterm infants with and without respiratory distress. A simple bedside method using nitrous oxide. *Pediatrics* 57 179 1973
- 26 Pacelt A F, Miller C F, Daily W J R & Savaglio F J Jr. Impedance technique for monitoring the newborn infant. *Digest of the 7th International Conference on Medical and Biological Engineering*, 1967 p 251
- 27 Pillet J E & Scopes J W. Recording respirations in newborn babies by measuring impedance of the chest. *Med Biol Eng* 3 161 1965
- 28 Patterson R, Kubicek W G, Kinnen E, Witsoe D & Noren G. Development of an electrical impedance plethysmograph system to monitor cardiac output. *Proceedings of the First Annual Rocky Mountain Bioengineering Symposium* 1964
- 29 Pomerantz M, Baumgartner M, Laundson J & Eiseman B. Transthoracic electrical impedance for the early detection of pulmonary edema. *Surveys* 66 260 1969
- 30 Pomerantz M, Delgado F & Eiseman B. Clinical evaluation of transthoracic electrical impedance as a guide to intrathoracic fluid volumes. *Ann Surg* 171 686 1970
- 31 Prec K J & Crissels D E. Dye dilution curves and cardiac output in newborn infants. *Circulation* 11 789 1955
- 32 Rudolph A M, Drorbaugh J E, Auld P A M, Rudolph A J, Nadas A S, Smith C A & Hubbell J P. Studies on the circulation in the neonatal period. The circulation in the respiratory distress syndrome. *Pediatrics* 27 551 1961
- 33 Selstam U & Olsson T. Monitoring with impedance plethysmography applied to newborn infants with disturbed functional adaptation. *Proceedings of the First Nordic Meeting on Medical and Biological Engineering*, Helsingfors 1970 p 25
- 34 Van De Water J M, Mount B E, Barela J R, Schuster M & Leacock F S. Monitoring the chest with impedance. *Chest* 64 597 1973

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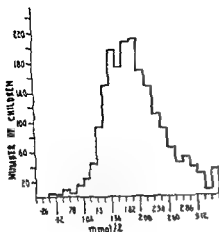


Fig 1 Frequency distribution for cord serum total cholesterol

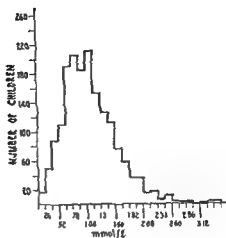


Fig 2 Frequency distribution for cord serum low density lipoprotein cholesterol

serum and at follow up for the control and HC groups are shown in Table 1. Seventy three HC neonates were followed up and 373 control group children were seen once or more during the first year. At follow up during infancy there were no statistically significant differences in the mean values for either TC or LDL C between any of the HC groups and the control group.

HC during infancy was defined as a serum TC and/or LDL C above the age adjusted 97.5 percentile which lay at 5.9 to 6.0 mmol/l for TC from 3 to 12 months and 4.26 mmol/l for LDL C at one year. The 97.5 rather than the

95th percentile was selected because it was considered most probable that children with genetically transmitted HC would fall in the higher range. 310 parents of the control group children had their serum cholesterol measured and from these samples the mean \pm S.D. and 97.5 percentile (mmol/l) values for TC and LDL C were defined for fathers: TC 5.17 ± 1.23 , 7.93; LDL C 3.08 ± 0.99 , 4.81; for mothers: TC 4.69 ± 0.94 , 6.80; LDL C 2.7 ± 0.93 , 4.81. 6.50 mmol/l lay at the 88th percentile for fathers and 95th percentile for mothers. Of the 73 neonates who had had cord serum HC who were reviewed during

Table 1 Values for TC and LDL C (mean \pm S.D. mmol/l) in cord serum and at follow up of the control group children (at one year of age) and of those with cord serum HC (groups 1 to 3—see text)

Group	Cord lipids	Cord serum ($\bar{x} \pm$ S.D. mmol/l)			Follow up value ($\bar{x} \pm$ mmol/l)		
		TC	LDL C	(n)	TC	LDL C	(n)
1	LDL C	9.0 \pm 1.7	10.0 \pm 1.6	37	4.31 \pm 0.93	3.1 \pm 0.98	71
	TC	3.39 \pm 0.88	3.1 \pm 0.57	49	4.37 \pm 1.0	2.39 \pm 1.07	28
2	LDL C	3.04 \pm 0.19	1.43 \pm 0.8	36	4.36 \pm 0.75	2.59 \pm 1.77	4
	TC	1.79 \pm 0.47	0.87 \pm 0.4	330	4.39 \pm 0.86	7.33 \pm 0.84	77

SAMPLE AND METHODS

The sample population comprised 2000 unselected babies born consecutively over a seven month period at the Queen Victoria Hospital Adelaide. Individual details concerning the pre and perinatal experiences of the babies were not recorded on delivery for logistic reasons but were retrospectively recorded from the clinical notes of the babies who were subsequently followed up. These details included the birth weight, the duration of gestation, the duration of labour, signs of intra partum fetal distress, Apgar scores at 1 and 5 min and whether resuscitation was required.

The umbilical cord was routinely clamped within 1 min of delivery and 5 to 10 ml of mixed blood was taken from the plicental end of the cord prior to delivery of the placenta. The serum was separated and stored at 4°C within 12 hours of delivery.

Biochemical analyses Cord blood high density lipoprotein (HDL C) was prepared by precipitation of other lipoproteins with heparin manganese chloride (7). The final concentration of heparin solution was 183 anti coagulability units/ml which resulted in complete precipitation of non HDL lipoproteins (5). This was confirmed by the disappearance of non HDL lipoprotein bands on polyacrylamide gel electrophoresis following heparin manganese precipitation. The HDL C fraction and the second aliquot of serum were frozen at -20°C prior to lipoprotein assay with the HDL C fraction being centrifuged to remove the particulate matter which appeared on thawing. Unpublished comparative studies in our laboratory have demonstrated no difference between the values for HDL C obtained on fresh serum or after freezing, thawing and centrifugation of the previously precipitated sample.

Serum TC and HDL C were measured using the enzymatic method of Allyn (1) and the reagent manufactured by Abbott Laboratories (Diagnostic Division, 820 Mission St, So Pasadena, CA 91030). Analyses were performed on a Centri Chem (Union Carbide) distributed by Hoffmann La Roche (Bisely) using 10 µl serum. A reference serum supplied by the Center for Disease Control Laboratory (Georgia) was used as a primary standard.

Artificially high values for HDL C which may occur if manual enzymatic methods are used (79) are not obtained when the enzymatic method is used on a centrifugal analyser (see appendix). The value of LDL C was derived using the formula $LDL C = TC - (TG/5 + HDL C)$. The results obtained in this way have been shown to correlate well with those obtained by ultracentrifugation (14).

Triglycerides (TG) were measured by an enzymatic method on Centri Chem on 10 µl serum using Calbiochem Triglyceride Glycerol Stat Pack (Calbiochem, California). A reference serum with an assigned value was used as a standard. The assigned value was determined using a manual enzymatic method (Calbiochem) with pure triolein as primary standard (8).

Duplicate quality control samples were included in each run to assess precision. One was placed in a fixed position, the other in a random position on the sample tray. There was no significant difference between

the results for quality control samples in fixed and random positions. The means values \pm standard deviation (mmol/l) and coefficients of variations (CV) for the quality control samples for TC were 1.87 ± 0.09 (CV 5.04%), 3.19 ± 0.09 (CV 2.9%) and for TG 0.249 ± 0.01 (CV 4.8%), 0.59 ± 0.02 (CV 3.6%).

Follow up studies Four groups were selected for follow up based on the cord serum levels of TC at 1 DL C group 1 LDL C only above the 95th percentile group 2 both TC and LDL C above the 95th percentile group 3 TC only above the 95th percentile group - the control population with cord serum TC and LDL C levels below the 95th percentile. The latter group was selected in a one in four sequence from birth order from the total sample population. Each mother was interviewed during her hospital stay, the purpose of the study explained and her cooperation sought. In the event of refusal the next child born at the hospital was selected for follow up. Three hundred and thirty of the 373 babies subsequently followed had an adequate cord sample specimen collected.

The children selected for follow up because of elevated serum lipid levels (groups 1, 2 and 3) were reviewed between 4 and 12 months of age. The children in the control population (group 4) were reviewed at the ages of 3, 6 and 12 months and a non fasting blood sample taken for lipid analysis. LDL C was measured at 12 months in control group children and on review of children who had cord HC. The lipid levels of parents were measured by the above techniques following a 12 hour fast.

Dietary and statistical analyses The average daily cholesterol and energy content of the diets were calculated from a combination of mothers' recall of their babies' intake over the preceding two days and a two day diet diary. Calculations were made using a standard source (10) and it was confirmed by analyses of representative brands of milk and infant foods (74). Statistical analyses were done by standard methods (25).

RESULTS

Of the 2000 babies included in the sample population, suitable serum specimens were obtained from 1926. One hundred and twenty nine specimens were insufficient for either HDL C and/or TG analysis so that 1797 results were obtained for LDL C. The values for the mean SD and 95th percentile (in mmol/l) were for TC 1.83 ± 0.56 , 2.86 for LDL C 0.90 ± 0.49 , 1.82 for HDL C 0.70 ± 0.33 , 1.27 for TG 0.38 ± 0.16 , 1.53 . These values are similar to previous studies. The frequency distribution curves for the cord serum TC and LDL C are shown in Figs 1 and 2 and the values (mean \pm SD) in cord

Table 2 Children with elevated cord serum TC LDL C and hypercholesterolaemia at follow up

Subject	Cord TC	Cord LDL C	3 months TC	6 months TC	1 year TC	1 year LDL C	Father		Mother		Family history early CHD
							TC	TG	TC	TG	
L R	96	182	478	570	640	575	481	181	413	095	Nil
1 S	99	179	450	632	468	786	512	076	387	055	Nil
V B	356	113	671	-	671	359	-	-	481	071	No history
T F	375	195	-	931	515	309	587	094	507	05	Nil
S B	317	211	-	601	637	421	637	107	523	102	P G G Fa
S M	768	190	-	593	637	471	780	311	494	-	No history

Hypercholesterolaemic parent (TC ≥ 6.5 mmol/l)

50 mg/day and another sub-group with an intake close to 100 mg/day

At 3 months of age 4% of the control group babies had a low cholesterol diet and 16.5% had a moderate cholesterol diet. By 6 months 59% had a low cholesterol diet 38.5% a moderate cholesterol diet and 2.5% a medium cholesterol diet. For the combined results of all the children reviewed in one year (groups 1-4 inclusive) the proportions had changed to low cholesterol diet 40% moderate cholesterol diet 46% and medium cholesterol diet 13%. No correlation was found between the serum cholesterol levels and dietary intake.

The possible influences of *intra partum* stress factors on cord serum lipoproteins was investigated in those 52 babies whose cord serum LDL C had been equal or greater than the 97.5th percentile of 1.97 mmol/l. This sub-group was arbitrarily selected for retrospective analysis as it was considered that the effects of adverse perinatal events on cord serum TC and LDL C would be evident in comparison with the control group. Among these 52 HC neonates the cord serum TC and LDL C was higher though not significant on testing for those with a birth weight less than 1500 g, a gestation period of less than 37 or greater than 42 weeks and those with a duration of labour greater than 11 hours. The mean values for cord TC and LDL C were the same or lower in comparison with those neonates who had not experienced these factors in

those whose labour had been induced who had had any documented sign of fetal distress whose one minute Apgar score was less than eight or who had required any resuscitative measure on delivery. No differences occurred in cord serum TG for any condition studied within the sample of 52 HC babies. Overall the adverse factors occurred significantly more frequently in the 52 HC neonates than in the NC control group (Table 3) $p < 0.01$.

DISCUSSION

This study was designed to test the hypotheses that an elevated cord serum LDL C might be a marker for genetically determined HC and that it might prove a more sensitive marker

Table 3 Percentage frequency of adverse fetal and perinatal events in control group children ($n=382$) compared with those who had a cord serum LDL C equal to or greater than the 97.5th percentile (HC group) ($n=52$)

	Control group	HC group
Birth weight <1500 g	3.4	34.6
Apgar 1 min <6	1.6	38.5
Apgar 5 min <8	7.8	15.3
Mother aged <16	4.7	5
Gestation <37 weeks or >41 weeks	11.5	34.6
Any sign of intrapartum fetal distress	9.2	13.5
Baby needed resuscitation	9	31

infancy HC was detected in six with one child (S M Table 2) having an HC parent. S B's father had moderate HC (6.37 mmol/l, 86th percentile) and his brother also had HC (TC 6.38 mmol/l); their paternal grandfather having died from an infarct at 55 years. No family history of premature ischaemic heart disease was present in other families. S B, S M and V B had HC persisting from 3 or 6 months to 12 months. The other three children had subsequently normal TC levels and normocholesterolaemic (NC) parents. No information was available about V B's father or his family.

Among the children in the control group ten of 354 (2.8%) were HC at 3 months of age and all but one child were NC at 6 and 12 months. At 6 months 10 of 310 children (3.2%) were HC and all but one other child were NC at one year. At one year 8 of 273 (2.9%) were HC and all were NC on retesting within 6 weeks. Overall 27 children were HC during infancy with four having an HC parent (>6.5 mmol/l). None of these adults had a family history of early ischaemic heart disease but two were overweight males with mixed hypertriglyceridaemia and moderate HC.

False positives and negatives The follow up of the HC neonates was incomplete because of lack of interest of the parents or the families having moved with no forwarding address given. The figures for the false negative and false positive results can therefore only present an approximation of the true picture. One (S M) of the 73 children HC as neonates was HC at follow up and had an unequivocally HC parent giving a minimal positive detection rate of 0.05% (1/1926). The false positive rate referring to those children with cord HC and subsequent NC was 3.7% (71/1926). False negative rates refer to those in whom the serum TC and/or LDL C was normal in cord blood but elevated at follow up; this was 1.1% (4/373).

Because of the low case finding rate the question was also asked: How many of the

parents had probable FH? Fourteen of the parents tested had a TC and/or LDL C above the 97.5th percentile for sex and of these parents seven had a significant family history of premature coronary heart disease (CHD) with a parent or grandparent having had an infarct under 65 years of age. Three of these parents had one or more such relatives affected in one generation, two had had both an affected parent and grandparent and two fathers had had an infarct and had both an affected father and grandfather (the child's great grandfather). None of these parents had children who were HC at one year.

Non attenders There were 114 mothers and babies in the control group who failed to attend at the 3 month and 6 month visit and whose babies had not been placed in category 1, 2 or 3. A randomly selected sub-group of 30 mothers was visited and the reason for default ascertained. Half the mothers either refused or had no forwarding address. Six lived too far distant, 4 had had their babies adopted, 1 baby had died and language and transport difficulties accounted for the remainder. A young mobile population with a high proportion of recent non English speaking migrants is characteristic of many suburban areas of Australia and the reasons given would also account for the failure of the parents of children in categories 1 to 3 to attend for follow up. In addition dietary questionnaires were completed by the visiting health worker and these excluded the possibility of non attending children being given a diet qualitatively different from those children seen at follow up.

Dietary analysis The mean daily dietary cholesterol intake was recorded as either a low cholesterol diet (0–100 mg cholesterol intake/day), a moderate cholesterol diet (101–250 mg cholesterol intake/day) or a high cholesterol diet (501–750 mg cholesterol intake/day). In retrospect the 100 mg dietary cholesterol/day division may have been set too high for inclusion in that group was a sub-group of children whose intake was less than

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							TC	TG	TC	TG	
L R	296	18	478	570	640	575	481	181	413	095	Nil
I S	99	~79	450	637	468	286	51	076	387	055	Nil
V B	346	213	671	-	621	359	-	-	481	071	No history
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							TC	TG	TC	TG	
L R	2.96	1.8	4.78	5.70	6.40	5.75	4.81	1.81	4.13	0.95	Nil
I S	.99	.79	4.50	6.37	4.68	2.86	5.17	0.76	3.82	0.55	Nil
V B	3.16	2.13	6.71	-	6.21	3.49	-	-	4.81	0.71	No history
T F	3.3	1.95	-	9.31	5.15	3.09	5.8	0.94	5.07	0.57	Nil
S B	3.17	2.11	-	6.01	6.37	4.71	6.37	1.07	5.73	1.07	P & G Fa
S M	2.68	1.90	-	5.93	6.37	4.21	7.80	3.11	4.94	-	No history

Hypercholesterolaemic parent (TC ≥ 6.5 mmol/l)

50 mg/day and another sub-group with an intake close to 100 mg/day

At 3 months of age 4% of the control group babies had a low cholesterol diet and 16.5% had a moderate cholesterol diet. By 6 months 59% had a low cholesterol diet 38.5% a moderate cholesterol diet and 2.5% a medium cholesterol diet. For the combined results of all the children reviewed at one year (groups 1-4 inclusive) the proportions had changed to low cholesterol diet 40% moderate cholesterol diet 46% and medium cholesterol diet 13%. No correlation was found between the serum cholesterol levels and dietary intake.

The possible influences of *intra partum* stress factors on cord serum lipoproteins was investigated in those 52 babies whose cord serum LDL C had been equal or greater than the 97.5th percentile of 1.97 mmol/l. This sub-group was arbitrarily selected for retrospective analysis as it was considered that the effects of adverse perinatal events on cord serum TC and LDL C would be evident in comparison with the control group. Among these 52 HC neonates the cord serum TC and LDL C was higher though not significant on *t* testing for those with a birth weight less than 2500 g, a gestation period of less than 37 or greater than 42 weeks and those with a duration of labour greater than 11 hours. The mean values for cord TC and LDL C were the same or lower in comparison with those neonates who had not experienced these factors in

those whose labour had been induced who had had any documented sign of fetal distress whose one minute Apgar score was less than eight or who had required any resuscitative measure on delivery. No differences occurred in cord serum TG for any condition studied within the sample of 52 HC babies. Overall the adverse factors occurred significantly more frequently in the 52 HC neonates than in the NC control group (Table 3) $p < 0.01$.

DISCUSSION

This study was designed to test the hypothesis that an elevated cord serum LDL-C might be a marker for genetically determined EC, and that it might prove a more sensitive

Table 3 Percentage frequency of perinatal events in control and hypercholesterolaemic neonates ($n=382$) compared with those whose cord serum LDL C equal to or greater than the 97.5th percentile (HC) ($n=52$)

Perinatal event	Control (%)	HC (%)
Birth weight < 2500 g	1.3	10.2
Apgar 1 score < 6	0.5	3.8
Apgar 5 score < 9	0.5	3.8
Mother age < 16	0.5	3.8
Gestation < 37 weeks	0.5	3.8
or > 41 weeks	0.5	3.8
Any sign of fetal distress	0.5	3.8
Fetal distress	0.5	3.8
Baby needed resuscitation	0.5	3.8

for future HC than cord serum TC alone and to determine the proportion of HC infants who had had normal cord serum lipoprotein levels. Particular emphasis was placed during the design of the study on ensuring a suitably large normal control population for prospective follow up a sequential analysis of LDL C in as many cord serum samples as possible and the use of cut off points for cord blood TC and LDL C derived from the 95th percentiles of the distribution curves of the actual sample.

Dominantly inherited FH can be reliably diagnosed clinically only with evidence of transmission through three generations of hyper low density lipoproteinemia and/or the presence of tendon xanthoma. However because of sociological factors in the sample reported here it was only possible to obtain circumstantial evidence of three generation transmission from a history of premature coronary heart disease (CHD) and HC in parent and child.

In this study no differences were found at follow up during infancy for the mean values of either TC or LDL C between those children who had been HC or NC at birth. Of the six children with cord serum HC and who were HC during infancy only one (S M) had a parent with unequivocal HC. Another child S B had circumstantial evidence of FH with his father and brother having moderate HC (6.37 mmol/l) and the paternal grandfather having died from an infarct at age 55 years. S M's father had marked HC (7.8 mmol/l) with no history of early CHD. This high rate of false positive values the minimum estimate being 3.7% may partly be explained by the influence of adverse perinatal factors which may cause an elevation in fetal serum cholesterol and TG levels (6.9.26). Although the incidence of adverse factors such as short gestation, low birth weight and signs of intrapartum stress were lower than in those with a cord serum LDL C greater than the 97.5th percentile amongst the latter group there were no differences in the mean values for TC, LDL C or TG in those who had or had not experienced

such an event. The results therefore suggest that the individual perinatal experience of a child needs to be considered when assessing his/her cord serum lipoprotein level.

The results of this study also showed that not only did those children who had been HC as neonates have similar serum cholesterol values to those who had been NC as neonates when followed during infancy but that transient HC was a common finding during the first year. Amongst those in the control group altogether 74% were HC at 3.6 or 12 months of age. This tendency to revert towards a normal serum cholesterol level was also described by Darmady (10) in her study. All of her 22 HC one year olds had lower levels after a two month interval. None of the parents of these control group children who became HC during infancy had the characteristics of FH with only four of these parents having moderate HC with a TC between 6.25 and 6.5 mmol/l. Two of these parents were young and obese fathers with mixed hypertriglyceridaemia and hypercholesterolaemia the other two parents being healthy with no family history of early CHD. However there was strong circumstantial evidence of FH in the families of two NC one year olds on the basis of paternal HC and early infarct and experience of premature CHD in these generations and in a further two families on the basis of parental HC and CHD through two generations.

During the first few months of life the dietary cholesterol load may be an important influence on the serum cholesterol level (12.13) with individual variation occurring between infants in the maturity of their homeostatic mechanisms (23) so that children with FH may be NC on a low cholesterol diet (31). Indirect evidence for the emergence of a more mature homeostatic state with regard to cholesterol regulation comes from unpublished data from the ongoing study of the control group of children with evidence for tracking for lipoproteins emerging during the second year and positive association then appearing

between the parents and children's lipoprotein fractions. Environmental factors thus may partly obscure the genetic influences on lipoproteins at least through infancy and prevent either the level of TC or LDL C in cord blood or during the first few months of life being predictive for future HC. The findings of this study are therefore in agreement with those of Darmady (10) and Potter (27) who both found cord HC unreliable as a marker for future HC and of Goldstein (16) who approached the problem from an epidemiological angle but have also shown that cord LDL C does not improve the validity of cord blood screening. The authors of other prospective studies including those of Glueck (15), Tsang (32), Greten (17) and Andersen (2) have concluded that children with FH may be detected by cord screening. Although we agree that this may be the case for individual families (21, 22, 33) and for children of parents affected with FH (20) we propose that this does not validate population cord screening programmes. We also suggest that information gained from this longitudinal study of a sample of normal children has provided useful information as to the pattern and extent of individual variability of cholesterol levels during infancy and has thus partly answered the doubts concerning the possible rates of false negative and false positive values in this screening procedure.

REFERENCES

- Allan, C. C., Ryan, L. S. & Chan, C. S. G. Richmond, W. & Fu, P. C. Enzymatic determination of total serum cholesterol. *Clin Chem* 20: 470 1974.
- Anderson, G. E. & Fris-Hansen, B. Neonatal diagnosis of familial type II hyperlipoproteinaemia. *Pediatr Res* 17: 714 1976.
- Anderson, G. E. & Nielsen, H. G. Neonatal screening for hyperlipoproteinaemia. Methods for direct estimation of cord serum VLDL & LDL. *Clin Chim Acta* 66: 79 1976.
- Anderson, G. E. & Fris-Hansen, B. Neonatal hypertriglyceridaemia. *Acta Paediatr Scand* 65: 369 1976.
- Backonck, P. S., Wood, P. D., Albers, J. J., Steiner, P., Dempsey, M., Kuba, K., Warnick, R. & Karlsson, L. Plasma high density cholesterol concentrations determined after removal of other lipoproteins by heparin/manganese precipitation or by ultra centrifugation. *Clin Chem* 27: 18 8 1976.
- Boulton, T. J. C. Perinatal stress and its effects on fetal lipoproteins. *Aust NZ J Med* in press.
- Burstein, M. & Samaille, J. Sur un dosage du cholestérol hématique et aux β lipoprotéines du sérum. *Clin Chim Acta* 5: 609 1960.
- Chong, K. T. R. & McLaughlin, P. Fully automated all enzymatic method adapted in Gem sac centrifugal analyser with use of an aqueous trisolein standard. *Clin Chem* 20: 1454 1974.
- Cress, H. H., Shahan, R. M., Laffin, M. & Karpowicz, K. Cord blood hyperlipoproteinaemia and perinatal stress. *Pediatr Res* 11: 19 1977.
- Darmady, J. M., Fosbrooke, A. S. & Lloyd, J. K. Prospective study of serum cholesterol levels during the first year of life. *Br Med J* 11: 685 1977.
- Fredrickson, D. S. & Levy, R. I. Familial hyperlipoproteinaemia. In J. B. Stanbury, J. B. Wyngarden & D. S. Fredrickson (eds). *The metabolic basis of inherited disease*. McGraw-Hill, New York 1977. 3rd ed. p. 545.
- Fredrickson, D. S. & Breslow, J. L. Primary hyperlipoproteinaemia in infants. *Annu Rev Med* 24: 315 1973.
- Friedman, G. & Goldberg, S. J. Concurrent and subsequent serum cholesterol of breast fed and formula fed infants. *Am J Clin Nutr* 28: 4 1975.
- Glueck, C. J., Steiner, P. & Leuba, V. Cord blood low density lipoprotein cholesterol estimation versus measurement with the preparative ultra centrifuge. *J Lab Clin Med* 82: 467 1973.
- Glueck, C. J., Heckman, F., Schoenfield, M., Steiner, P. & Pearce, W. Neonatal familial type II hyperlipoproteinaemia. cord blood cholesterol in 1800 births. *Metabolism* 20: 597 1971.
- Goldstein, J. L., Albers, J. J., Schrott, H. G., Hazzard, W. R., Berman, E. L. & Motulsky, A. G. Plasma lipids levels and coronary heart disease on adult relative of newborns with normal and elevated cord blood lipids. *Am J Hum Genet* 26: 727 1974.
- Greten, H., Wengeler, H. & Wagner, H. Early diagnosis of familial type II hyperlipoproteinaemia. *Nur Metab* 15: 178 1973.
- Kannel, W. B. Prospects for prevention of atherosclerosis in the young. *Aust NZ J Med* 11: 410 1976.
- Kwiterovich, P. O., Levy, R. I. & Fredrickson, D. S. Early detection and treatment of type II hyperlipoproteinaemia. *Circulation* 67: 37 1970.
- Kwiterovich, P. O., Levy, R. I. & Fredrickson, D. S. Neonatal diagnosis of familial type II hyperlipoproteinaemia. *Lancet* i: 118 1973.
- Lee, G. B., Culley, G. A., Lawson, J. J., Adcock, L. L. & Knivett, W. Type II hyperlipoproteinaemia in mother and twins. *Circulation* 39: 183 1969.
- Lewis, L. A., Brown, H. M. & Green, J. G. Serum cholesterol and lipoprotein levels of familial hypercholesterolaemic infants. *Circulation* 35: 74 1967.
- Nestel, P. J., Poyser, A. & Boulton, T. J. C. Cholesterol homeostasis in infants. *Proc Aust Soc Med Res* 10: 55 1977.
- Nestel, P. J. Personal communication.

- 25 Nie N H, Hull C H, Jenkins J G, Steinbrenner K & Bent D H. *Statistical package for the social sciences*. McGraw Hill, New York 1975
- 26 Potter J M. Perinatal plasma lipid concentrations. *Aust NZ J Med* 7: 155 1977
- 27 Potter J M & Nestel P J. Comparison of plasma lipids at birth and in second year of life. *Aust NZ J Med* 6: 470 1976
- 28 Slack J. Risks of ischaemic heart disease in familial hyperlipoproteinemic states. *Lancet* II 1380 1969
- 29 Steele H W, Kockler D F, Aver M M, Blanz K, Koworko T P, Kuber K & Dempsey M E. Enzymatic determinations of cholesterol in high density lipoprotein fractions prepared by a precipitation technique. *Clin Chem* 22: 98 1976
- 30 Thomas W & Corden M. *Tables of composition of Australian foods*. Nutr Committee N H & M R C Aust Commonwealth Dept Health 1970
- 31 Tsang R C, Fallat R W & Glueck C J. Cholesterol at birth and age 1: comparison of normal and hypercholesterolemic neonates. *Pediatrics* 53: 458 1974
- 32 Tsang R C, Glueck C J, Fallat R W & Mellies M. Neonatal familial hypercholesterolemia. *Am J Dis Child* 129: 83 1975
- 33 Wolff O H. Familial hyperlipidaemias. *Proc R Soc Med* 60: 1147 1967

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TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

II Relation to Activity and Body Temperature

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ABSTRACT Hammarlund K, Nilsson G E, Öberg P Å and Sedin G (Department of Paediatrics, University Hospital, Uppsala and Department of Biomedical Engineering, University of Linköping, Sweden). Transepidermal water loss in newborn infants. II. Relation to activity and body temperature. *Acta Paediatr Scand* 68: 371, 1979. —Using a method described in a previous article the transepidermal water loss (TEWL) was studied in 10 healthy newborn infants at rest and during activity. On the average TEWL was 37% higher during activity than during rest although no sweating was observed. In 9 infants placed in incubators with an ambient temperature slightly above the thermoneutral range measurements were made as the body temperature rose. TEWL was almost constant until a temperature of 37.1°C was reached whereupon the water loss suddenly increased as the infant started sweating.

KEY WORDS Water loss, water balance, newborn infants, neonatal intensive care, temperature regulation.

The effect of activity on insensible water loss (IWL) has been studied by several authors (3, 5, 11) but it has been difficult to distinguish the amount of water lost from the skin from the water loss during respiration. In studies of insensible water loss in relation to body temperature Hey & Katz (3) have found a marked increase in water loss when the body temperature was above 37.2°C. These measurements included water lost from the respiratory tract and from skin areas with both eccrine and apocrine sweat glands. In previous studies it has not been possible to discriminate between the effects of activity and increasing body temperature on IWL, as a change in one often induces a change in the other.

With the method and measurement procedure described earlier (2, 5, 7, 8) the transepidermal water loss can be studied during varied activity at a constant ambient temperature and humidity and with only minor changes in body temperature, measured as deep rectal temperature. It is also possible to study the

effect of rising body temperature in an ambient temperature slightly above the thermoneutral range without concomitant changes in activity.

In our earlier studies (2, 8, 9) we have avoided the effects of physical activity and high body temperature by making all measurements with the infant at rest and with a body temperature of 37°C or below. The purpose of this study was to investigate the transepidermal water loss in relation to rest and activity and in relation to changes in body temperature.

SUBJECTS

Measurements of the evaporation rate (ER) were performed on 19 healthy newborn infants born after 37 to 39 weeks of gestation. All infants were appropriate for gestational age. The measurements were performed during the first 74 hours after delivery. The infant's skin was neither washed nor wiped before the measurements. In 10 infants ER was measured during rest and activity (Table 1) and in 9 infants placed in an ambient temperature slightly above the thermoneutral range (4, see Table 2) ER was measured during an increase in body temperature (Table 2).

Table 1 Infant data and measurement conditions in the activity study

Seven infants were delivered by Caesarean section Seven infants were male three female \bar{x} \pm SD = standard deviation

Infant	Gestational age (completed weeks)	Weight at birth (kg)	Length at birth (m)	Age at start of measurement (h)	Duration of measurement (h)	T_{sk1} (°C)			T_{rect} (°C)		
						Before activity	During activity	After activity	Before activity	During activity	After activity
1	39	3.680	0.530	1.5	1.9	35.0	35.2	35.0	36.7	36.8	36.8
2	38	3.070	0.470	2.0	2.6	35.1	35.7	35.1	36.9	36.9	36.9
3	38	3.730	0.520	3.0	1.5	35.5	35.8	35.8	36.6	36.6	36.6
4	38	3.670	0.525	1.7	1.8	36.2	36.2	36.0	36.5	36.7	36.1
5	39	2.850	0.500	1.3	1.9	34.8	35.3	34.9	36.2	36.4	36.5
6	38	3.990	0.510	1.3	1.4	36.3	36.3	36.0	36.8	37.0	37.0
7	38	3.750	0.500	2.7	1.5	35.7	35.8	35.7	36.7	36.7	36.7
8	38	2.910	0.490	2.2	1.3	35.6	35.8	35.9	36.7	36.7	36.8
9	38	3.770	0.500	1.1	1.3	35.4	35.6	35.5	36.3	36.4	36.4
10	39	2.960	0.485	2.6	1.5	36.0	35.8	35.9	36.5	36.7	36.7
Mean	38.3	3.478	0.503	1.9	1.7	35.6	35.8	35.6	36.6	36.7	36.7
\pm SD	0.5	0.437	0.019	0.7	0.4	0.5	0.1	0.4	0.2	0.2	0.1

METHODS

The rate of evaporation from the skin (ER) was measured by a method based on determination of the vapour pressure gradient in the air layer close to the skin surface (6, 7, 8). The method, which has been used earlier in studies on water loss at varying ambient humidities in full term infants (2) and in low birth weight infants (9, 10), allows accurate measurements without disturbing the activity of the infant. The equipment for measurement of ER also gives data on the ambient humidity (RH_{amb}) and ambient vapour pressure ($P_{H_2O, amb}$). The ambient air temperature (T_{amb}), skin temperature (T_{sk1}) and body temperature (T_{rect}) were measured with a YSI tele thermometer (43 TA and 4002 using probes 405, 421 and 402, Yellow Springs, Ohio, USA). Recordings were made with a Watanabe recording system (Watanabe Instruments Corp., Tokyo, Japan).

MEASUREMENT PROCEDURE

All measurements were made with the infant in an incubator (AGA MK41 or MK241, AGA Medical, Lidköping, Sweden) and with an inflow of air to the incubator of 8 l/min (cf. 2). The infants were naked and were placed in the lateral position. The ambient relative humidity was kept at 50%. The ambient temperature was measured in the centre of the incubator. Body temperature was measured as deep rectal temperature and skin temperatures were recorded from the skin areas where the ER measurements were made.

1 Estimation of transepidermal water loss in relation to activity

ER was measured on the chest (a) on an interscapular skin area (b) and on a buttock (c). The transepidermal

Table 2 Infant data and measurement conditions in the body temperature study

All infants (four male, five female) were delivered by Caesarean section \bar{x} \pm SD = standard deviation

Infant	Gestational age (completed weeks)	Weight at birth (kg)	Length at birth (m)	Age at start of measurement (h)	Duration of measurement (h)	T_{sk1} (°C)		T_{rect} (°C)		T_{mb} (°C)	
						range	\bar{x}	range	\bar{x}	\pm SD	\pm SD
1	38	3.400	0.505	1.1	2.7	35.6–36.8		36.0–37.4		36.9 \pm 0.2	
2	39	3.120	0.480	0.5	3.0	35.0–36.5		35.9–37.3		35.8 \pm 0.4	
3	38	3.860	0.535	0.5	2.3	35.6–36.4		36.1–37.2		36.6 \pm 0.3	
4	38	3.670	0.505	0.5	3.8	35.4–36.4		36.2–37.2		36.2 \pm 0.4	
5	39	2.650	0.480	0.5	2.3	35.5–36.5		36.1–37.2		35.7 \pm 0.2	
6	38	3.000	0.470	0.6	4.1	34.7–36.4		35.8–37.3		35.3 \pm 0.2	
7	39	3.260	0.500	0.6	1.8	34.8–35.9		36.4–37.2		35.1 \pm 0.1	
8	38	4.200	0.570	0.6	2.7	34.7–36.3		36.4–37.2		35.1 \pm 0.1	
9	39	3.810	0.570	0.6	2.4	34.5–36.2		36.3–37.2		35.2 \pm 0.2	
Mean	38.4	3.441	0.502	0.6	2.8					35.8	
\pm SD	0.5	0.487	0.027	0.2	0.7					0.7	

m (°C)

Before activity	During activity	After activity
37.8	37.8	37.8
37.4	33.5	33.4
37.0	35.3	36.0
37.0	34.0	34.0
37.7	33.3	33
37.8	35.9	35.6
37.7	33.5	33.7
37.5	33.5	33.0
37.8	33.7	33.6
37.4	34.3	34.1
37.9	34.0	33.9
37.9	34.0	33.1

water loss (TEWL, g/m² h) i.e. cutaneous water loss per unit area was calculated as described previously (2) using the equation

$$TEWL = 0.9 \cdot ER_{sk} \cdot \Delta T_{sk} + 1.37$$

T_{sk} was kept as constant as possible and T_{mb} , T_{sk} and T_{hd} were measured at the same times as ER (Table 1)

At the beginning of each measurement series the infant was asleep and had been calm without crying for at least 10 min. When measurements had been made at all three measurement points the infant was made to cry for 10 min by intermittent gentle tactile stimulation at the end of which period measurements were made with the infant crying. The procedure was terminated with measurements after a period of at least 10 min during which the infant was asleep or if awake was quiet and showed little motor activity. Thus values for TEWL were obtained in each infant before, during and after activity (crying).

Estimation of transepidermal water loss in relation to body temperature

Within 30 min of birth the infant was placed in an incubator with an internal temperature (T_{mb}) slightly above the thermoneutral range (4, see Table 2). With the infant at rest ER was measured on the chest (a), on an inter-scapular skin area (b) and on a buttock (c) and TEWL was calculated. Repeated measurements were made while T_{hd} rose. T_{mb} was kept as constant as possible. T_{sk} and T_{hd} were recorded for each measurement point. The measurements were terminated when an increase in TEWL was obtained or when the infant showed obvious signs of discomfort (tachypnoea, vomiting).

TREATMENT OF DATA

Data were thus obtained for ER, RH, P_{H_2O} , T_{sk} , T_{hd} and T_{mb} .

After calculation of TEWL for different T_{hd} values for TEWL at equidistant temperatures (0.1°C) were ob-

tained by linear interpolation between the actual measurement values for each individual infant. The arithmetic mean of the three measured skin temperatures for each TEWL was designated T_{sk} (Tables 1 and 2). All statistically significant differences reported in the following were obtained by testing paired observations (Student's *t* test).

RESULTS

Transepidermal water loss in relation to activity

The mean TEWL before activity was 5.3 ± 1.1 (S.D.) g/m² h. During activity an increase to 6.4 ± 1.8 (S.D.) g/m² h was observed. The mean TEWL after a period of rest following activity was 3.9 ± 0.6 (S.D.) g/m² h (Fig. 1).

Thus a mean increase in TEWL of 20 ± 14 (S.D.) % was obtained when the values before and during activity were compared in each infant. After the period of activity the mean TEWL decreased and values lower than those before activity were obtained (Fig. 1). The mean TEWL during activity was 37 ± 23 (S.D.) % higher than the mean value for the periods of rest in each infant.

In the infants with a high TEWL before activity TEWL was also high during activity and in most cases also after activity.

During activity the mean T_{skin} was 0.2°C higher than during the periods of rest ($p < 0.05$). The mean T_{body} was 0.1°C higher both during and after activity than before activity ($p < 0.01$).

Transepidermal water loss in relation to body temperature

In Fig. 2 the TEWL values for each infant (left part) and the mean TEWL values (right

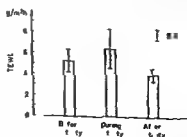


Fig. 1 TEWL before, during and after activity in 10 newborn infants. S.D. = standard deviation.

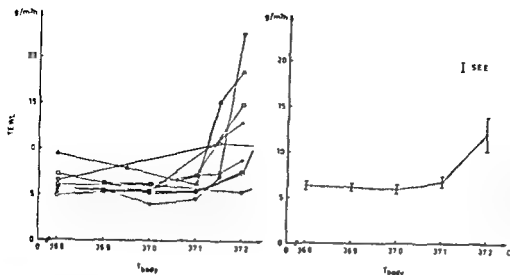


Fig. 2. TEWL in relation to body temperature in 9 newborn infants. Individual values on the left; mean values on the right. SEE = standard error of estimate.

part) are given in relation to T_{body} . A rise in TEWL occurred when T_{body} was around 37.2°C. As can be seen in the right part of the figure small SEE were obtained in the T_{body} range of 36.8–37.1°C. When T_{body} rose from 37.1 to 37.2°C the mean TEWL increased by nearly 80% and scattering of the individual TEWL values occurred. The absolute TEWL values at T_{body} higher than 37.2°C are of limited relevance according to the definition of TEWL (cf. 2 and Discussion).

DISCUSSION

In studies of transepidermal water loss in newborn infants it has been difficult to distinguish between effects of environmental factors, gestational age, body temperature and activity. The only way to obtain better knowledge of TEWL is to investigate each factor separately while the others are kept as constant as possible.

Our previous studies have been made with the infant at rest with a normal body temperature and in a constant environment within the thermoneutral range (4). The air velocity in the incubator has been kept low. Measurements have been made with a 50% relative humidity except when the effects of environmental humidity have been examined. As during these studies changes in activity and body temperature have been noted to affect TEWL, a systematic study of the effect of changes in ac-

tivity and body temperature has been considered necessary.

In the present study TEWL was 37% higher during activity than during periods of rest. This difference exceeds the increase in insensible water loss (IWL) reported by Hey & Kitz (3). In IWL respiratory water loss is included. The finding of Zweimüller & Preinin (11) that IWL could increase by a factor of at least 1.7 during periods of activity implies that the respiratory water loss increases greatly with crying and/or motor activity. Further comparisons with earlier studies are not valid as either the duration or the degree of activity (3, 5) or the environmental humidity (5, 11) have not been specified exactly.

Disturbances of the sleeping newborn infant cause increased motor activity and crying. It is probable that motor activity in the form of movements and crying gives rise to increased heat production in the working muscles which would lead to an increased body temperature if the thermoregulation of the infant did not intervene. The means available for cooling are increased blood flow through the skin and activation of the sweat glands.

In this study all infants showed a visible increase in skin perfusion during activity while no visible sweating was observed. The small changes in skin temperature in this study can only have contributed to a minor part of the increase in TEWL during activity by raising the diffusion rate of water through the skin. It

therefore seems probable that a considerable part of the increase in TEWL was mediated through the sweat glands by the combined effect of changes in the local skin temperature and activation of the central thermoregulatory system (cf 1)

The fact that the TEWL was lower in the period of rest after activity than before activity may be partly explained by age related changes in TEWL in this early period of life (see Table 1). This has also been observed in some of our other studies and will be discussed further in a future article. Although no differences were observed in the state of rest before and after activity a contribution of such effects cannot be excluded.

In the body temperature range 36.0–37.0 °C the activity of the newborn infants could be kept constant and low while the body temperature increased. This was also true up to a body temperature of 37.2 °C but at this point visible sweating occurred and was in most infants followed by an increase in activity, flushing and an increase in the respiratory rate. Thus at higher body temperatures the effects of body temperature and activity on the TEWL could not be kept separate in this study. Such changes in activity with increasing body temperature have not been reported in other studies (3).

The equation for calculating TEWL was based on data obtained in the body temperature range 36.0–37.0 °C and the effects that could have been caused by the uneven distribution of the apocrine sweat glands were thereby avoided. The TEWL obtained at a T_{body} of 37.2 °C underestimates the actual cutaneous water loss. Further at this body temperature the TEWL given in Fig. 2 cannot be considered as the maximum value as sweating had not reached a steady state.

According to Benzing (1) the human thermostat is situated in the hypothalamus and works with a temperature set point that controls sweating. Deep rectal temperature does not allow conclusions to be drawn as to the set point in adults as the changes in rectal

temperature are fairly slow. Tympanic temperature and oesophageal temperature reflect the hypothalamic temperature more accurately. The differences between rectal and tympanic temperatures and their relation to the induction of sweating have not come within the frame of this study. In infants with high activity it was observed that sweating was obtained at a somewhat lower body temperature than in infants at rest. These infants have not otherwise been included in this report or in Fig. 2 and Table 2. Induction of sweating at a lower deep rectal temperature may be the result of a raised hypothalamic temperature in the more active infants.

CONCLUSIONS

Under the conditions in which this study was performed it was concluded that

1. Transepidermal water loss increases considerably with activity without visible sweating or appreciable increases in deep rectal temperature.

2. Transepidermal water loss at rest increases markedly with a rise in deep rectal temperature above 37.1 °C.

ACKNOWLEDGEMENTS

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REFERENCES

1. Benzing T. H. Heat regulation. Homeostasis of central temperature in man. *Physiol Rev.* 49(4) 671 1969.
2. Hammarlund K., Nilsson G. E., Öberg P. Å. & Sedn G. Transepidermal water loss in newborn infants. I. Relation to ambient humidity and site of measurement and estimation of total transepidermal water loss. *Acta Paediatr Scand.* 66 553 1977.
3. Rev E. N. & Katz G. Evaporative water loss in the newborn baby. *J Physiol.* 200 605 1969.

- 4 Hey E N & Kitz G The optimum thermal environment for naked babies *Arch Dis Child* 45 328 1970
- 5 Little J A Brodsky W A & Greuthouse R The insensible weight loss of newborns and of older infants *Am J Dis Child* 90 630 1955
- 6 Nilsson G F Measurement of water exchange through skin *Med Biol Eng Comput* 15 209 1977
- 7 Nilsson G F On the measurement of evaporative water loss. Methods and clinical applications. *Linköping Studies in Science and Technology Dissertations No 11 and Linköping University Medical Dissertations No 48* Linköping 1977
- 8 Nilsson G Sedin G & Öberg Å A transducer for measurement of evaporation from the skin *Proc Internat Conference on Biomedical transducers* Paris Nov 3-7 1975 part II p 71
- 9 Sedin G Hammarlund K Nilsson G E & Öberg P Å Evaporative water loss in full term and pre mature newborn infants *Abstr No 47 6th Nordic Congress of Perinatal Medicine* Oulu Sept 15-17 1977
- 10 Sedin G Hammarlund K Nilsson G E & Öberg P Å The influence of environment activity and gestational age on evaporative water loss *Proc Annual Meeting of European Society for Paediatric Research No 51* Turku June 25-29 1978
- 11 Zweimüller E & Freimig O The insensible water loss of the newborn infant *Acta Paediatr Scand* Suppl 205 1970

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A FOLLOW UP STUDY OF INFANTS WITH ADVERSE REACTIONS TO COW'S MILK

I Serum IgE Skin Test Reactions and RAST in Relation to Clinical Course

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From the Department of Paediatrics and the Blood Centre University Hospital Uppsala Sweden

ABSTRACT Dannaëus A and Johansson S G O (Department of Pediatrics and the Blood Centre University Hospital Uppsala Sweden) A follow up study of infants with adverse reactions to cow's milk. I Serum IgE skin test reactions and RAST in relation to clinical course. *Acta Paediatr Scand* 68 377 1979—47 infants with cow's milk sensitivity were followed for a period varying between 6 months to 4 years (mean 28 months). The age at onset of symptoms varied between 11 days to 8 months. The clinical course was studied in relation to reagenic allergy by use of serum IgE skin prick test and RAST. Infants with an immediate onset of symptoms from the gastrointestinal tract and the skin after cow's milk intake were discerned as a distinct entity having a high frequency of atopy in the family. positive skin tests and positive RASTs to milk (71%). Cases with delayed reactions to cow's milk seldom had a positive RAST or skin test. Most infants of both groups showed an increasing tolerance to milk. In RAST positive infants the RAST titers increased significantly after onset of symptoms. After having reached a peak the titers subsided in several cases. The titers did not reflect the degree of milk sensitivity during the follow up period. However, in infants who developed high titers seemed to develop tolerance more slowly than infants with low titers.

KEY WORDS Atopic allergy infants IgE cow's milk allergy

Gastrointestinal, dermal, respiratory and anaphylactic reactions as well as a variety of other symptoms in infancy have been attributed to sensitivity to cow's milk (9). Lack of objective criteria for the diagnosis explains the varying incidence reported in the literature (0.3–7.5%) (1–5, 8). A reagenic allergy reaction (type I reaction) has been suggested in cases with rapid onset and short duration of symptoms (11–13) and a type III or IV reaction has been suggested in cases with delayed onset and often prolonged duration of symptoms (12, 16–17).

The diagnostic value of skin tests for milk antibodies belonging to different immunoglobulin classes are considered to be limited as positive tests are commonly found even in individuals tolerating milk (14–15, 6). Since sensitivity to milk is usually transient, a prospective study might elucidate some of the immunologic variables involved in the pathogenesis and the development of clinical tolerance.

The aim of the present investigation has been to study the clinical course of cow's milk sensitivity and its relationship to reagenic allergy by use of serum IgE skin prick test and RAST.

MATERIALS

The group under study consisted of 47 children, 26 boys and 21 girls, with cow's milk sensitivity who during 1974–77 were investigated at the Department of Paediatrics University Hospital Uppsala. The diagnoses were based on the following criteria:

1. Disappearance of symptoms after elimination of cow's milk from the diet.

2. Reappearance of symptoms within 24 hours following reintroduction of cow's milk. Repeated confirmatory challenge tests were performed in most children.

The term cow's milk sensitivity was used for all adverse reactions to cow's milk while the term cow's milk allergy is restricted to reagenic allergy.

In children with a clinical picture of malabsorption, disaccharidase deficiencies and coeliac disease were excluded by jejunal biopsies, lactose and xylose tolerance tests. Only two children exhibited abnormal biopsies and in both cases the diagnosis coeliac disease was verified by

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Complete tolerance	29	74

gluten provocation and repeated biopsies. In addition to the gluten sensitivity both children exhibited gastrointestinal reactions to cow's milk which remained despite a normal lactose absorption and a restored mucosa on a gluten free diet.

The children were divided into 2 groups according to the reaction onset time. 28 children who had symptoms within one hour following cow's milk intake were assigned to Group I and 19 children with a more delayed onset of symptoms (range 1-24 hours) to Group II.

The age at onset of symptoms varied from 14 days to 8 months in Group I and from 1 month to 20 months in Group II with an arithmetic mean of 3.3 and 4.9 months respectively.

A family history of allergic disease was obtained in 64% in Group I and 76% in Group II. 21% of the children in Group I had a double parental history of allergic disease compared to none in Group II.

The mean duration of breast feeding was 3.0 months in Group I and 2.3 months in Group II. When breast feeding was impossible as substitution for cow's milk a commercial soy bean formula Sobee[®] was used.

METHODS

The follow-up varied between 6 months and 4 years (mean 28 months) and 37 children were followed for more than 24 months. Thirty-five children came for a final examination including a blood sampling and skin prick tests with milk and soy bean preparations.

The median number of blood samples per child was 3 (range 1-8). In all children the first blood samples were obtained within 6 months and in one half of the children within one month after the onset of symptoms. The sensitivity to cow's milk was considered possibly decreased if according to parental estimates small amounts of milk in food stuffs were better tolerated than at the onset of symptoms and as definitely decreased if an increased amount of whole milk was tolerated.

Challenge tests in children with a case history of anaphylactic reactions after cow's milk intake were performed carefully with 1 ml of dilution 10⁻⁴ of fresh cow's milk as the initial dose. If no reaction was observed 1 ml

of 10 times less diluted cow's milk was given every hour. In a few extremely sensitive children challenge tests were considered too risky to perform. If there was no acute symptoms according to the case history and the skin test was negative 1 ml of undiluted cow's milk was given as initial dose. In the absence of a reaction within 30 min 10 ml was given and 30 min later another 40 ml.

Skin tests were performed as prick tests and the reactions were observed after 20 min. A wheal size of 3x3 mm or more was considered positive (2). A reaction similar to or larger than the wheal obtained with a histamine solution (1 mg/ml) was regarded as strongly positive (+++) and intermediate reactions as (++) in children less than one year of age. Tests giving no wheal but an erythematous flare exceeding 5x5 mm were also considered positive (+). In children suspected to be extremely sensitive contact testing was performed with a drop of milk applied on the skin for 15 min. Wheal and flare reactions were considered a positive test. The parents were also asked to report delayed skin reactions appearing during 24 hours following the skin prick test.

IgE concentrations were measured by a radioimmunosorbent technique PRIST (4). The IgE antibody activity was determined by RAST (10) using ¹²⁵I labeled anti D⁹. The results were expressed in PRU/ml (Phadebas RAST units Pharmacia Diagnostics AB Uppsala) and concentrations greater than 0.2 PRU/ml were considered positive.

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Allergen	Positive	
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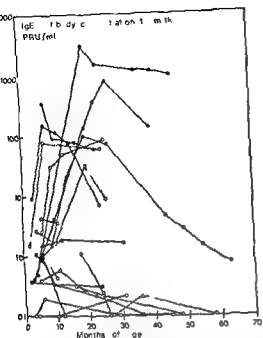


Fig 1 IgE antibody concentrations to milk. Milk RAST positive infants with unchanged or possibly decreased sensitivity (●) and with definitely decreased sensitivity or complete tolerance (○) at the end of the follow up.

RESULTS

In Group I one third of the children had onset of symptoms already by their first few meals of cow's milk formula. The most frequent symptoms were urticaria (71%) and diarrhoea (64%). The duration of symptoms varied from 2-24 hours. In Group II the onset of symptoms was generally more insidious and mainly confined to the gastrointestinal tract. Most children in this group developed a clinical picture of malabsorption.

Clinical course

During the follow up period several children had recurrent episodes of allergic symptoms even on a milk free diet.

In Group I asthma occurred in 25% and eczema in 43% but in Group II only one child had such symptoms. Furthermore Group I children had additional food sensitivities with reactions to egg (50%), fish (23%), pea (14%), soy bean (14%) and cereals (11%). In Group II

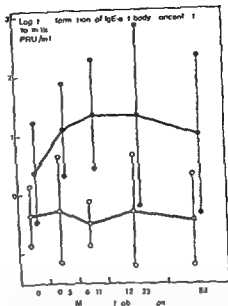


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Five children seemed to be sensitive enough to react to the trace amounts of cow's milk protein present in breast milk when the mother ingested milk. In two cases skin prick tests with the mother's breast milk were positive but turned negative when repeated with breast milk from the same mother off cow's milk. Most children in both groups tolerated increased amounts of milk at the end of the follow up and complete tolerance was found in 29% in Group I and 74% in Group II (Table 1).

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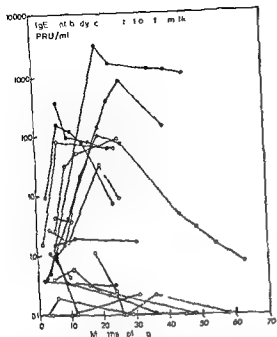


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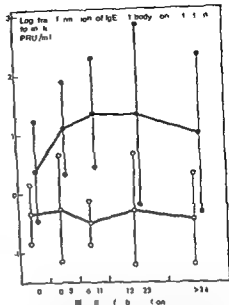


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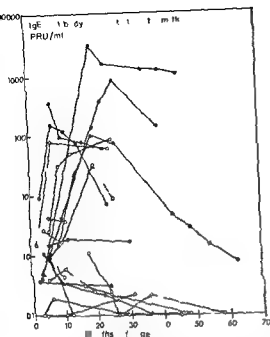


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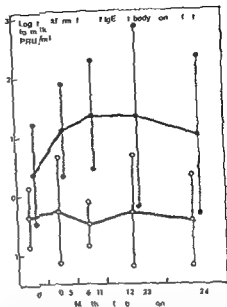


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Table 3 The relationship between the results of skin prick tests and RAST tests with milk and soy bean in 20 children of Group I at tendix, the final follow-up investigation

Allergen	Prick test result	RAST result			
		Negative	Positive		
			0.2-5 PRU	>5 PRU	
Milk	Negative	4	3	1	0
	+	2	1	1	0
	++	7	2	2	3
	+++	7	0	2	5
Soy bean	Negative	10	8	2	0
	+	2	1	1	0
	++	3	1	1	1
	+++	5	1	1	3

16.2 kU/l) ($p < 0.01$) and the levels increased further during the follow-up. Even slightly elevated IgE levels at onset seemed to be predictive of rising titers of specific IgE antibodies to milk.

Skin prick tests

At the onset of the follow-up period 68% of the children of Group I and 21% of Group II had positive skin prick tests with cow's milk.

At the final follow-up examination attended by 20 children of Group I and 15 children of Group II, the former showed an increased frequency of positive skin tests with milk and the reactions seemed to be stronger in most cases. The skin reactivity was greatest for BLG and greater for unboiled compared to boiled milk (Table 2). At the same time, 50% of the children in Group I had positive skin prick tests with soy bean including all four children who had symptoms such as eruption of eczema or increased itching after intake of a soy bean formula. In Group II only one child had a positive skin prick test with milk and none with soy bean. Three children, who originally were prick test positive with milk, were all negative and had developed complete tolerance to milk.

Delayed skin reactions were reported by the parents of Group II in 6 patients compared to one in Group I. Two of the children had erythema, swollenness and pruritus at the site of the puncture 2-4 hours after prick tests with BLG. Four children had similar reactions 2-17 hours after prick skin tests with soy bean, two of whom had diarrhoea provoked by a soy bean formula. None of the children with delayed reactions had immediate prick skin test reactions or a positive RAST.

RAST tests

At the onset of the investigation period 71.4% of the children in Group I had positive RAST tests to milk compared to 10.5% in Group II. The titers were significantly higher in Group I (arithmetic mean 19.6 PRU/ml, range 0.2-3300 PRU/ml) than in Group II (arithmetic mean 0.37 PRU/ml). There was no correlation between the duration of cow's milk feeding and the RAST titers. In children with a positive RAST to milk the titers increased significantly during the follow-up ($p < 0.05$) and even two children with initially negative RAST developed positive RAST to milk. The RAST titers often showed a peak and thereafter declined (Fig. 1).

During the investigation period the IgE antibody titers to milk were significantly lower in milk RAST positive children who developed complete tolerance or definitely decreased sensitivity ($p < 0.05$) compared to milk RAST positive children with unchanged or possibly decreased sensitivity (Fig. 2).

Positive RASTs to soy bean were found in 61% in Group I (arithmetic mean 19.0 PRU/ml, range 0.22-165 PRU/ml) and in only one patient in Group II (0.64 PRU/ml), one third of them already had positive RASTs at onset of the investigation period. All children had also positive RASTs to milk and the titers to soy bean developed in a similar manner to the titers to milk. The correlation of RAST to the skin prick test was good as shown in Table 3. One of the two children included in the

sensitivity had a normal serum IgE level negative skin prick tests and negative RAST. The other had an elevated IgE level (180 kU/l) and positive RAST to milk and soy bean.

DISCUSSION

The significance of reagins in cow's milk sensitivity has been disputed and the frequency of IgE antibodies to milk in this condition has varied considerably in different reports (3-7, 11). In this study IgE antibodies to milk were detected in high frequency in children with immediate type reactions in the skin and gastrointestinal tract after cow's milk intake. Several children developed additional sensitivity to other foods, animal danders and pollens and most children had a family history of allergic disease which indicate an atopic predisposition in this group.

It is now evident that atopic children may have IgE antibodies to milk without any obvious symptoms (6, 5). Therefore symptoms cannot be attributed to IgE antibodies alone. A variety of additional factors such as gut permeability, antibodies with blocking capacity, the stability of mast cells and the susceptibility of the shock organs to released mediators determine if symptoms will occur or not.

One third of the children in Group I had no detectable IgE antibodies to milk. This could indicate non reaginic mechanisms or the occurrence of IgE antibodies to allergenic determinants not present in the cow's milk preparation used or degradation products (18). Absence of IgE antibodies to milk in serum because of complete fixation of such antibodies to mast cells seems unlikely.

In this study as in others RAST and skin prick test reactions revealed IgE antibodies to milk with the same accuracy (2). One advantage of RAST is that potentially dangerous challenge tests can be avoided in patients who have shown anaphylactic reactions.

Most children developed an increased toler-

ance and none reported an increased sensitivity to cow's milk.

With a few exceptions children with low antibody levels developed tolerance more rapidly than those with high levels. Such a form of low degree transient cow's milk allergy is probably common and often overlooked (19). However the initial milk IgE antibody titers short after onset did not seem to be of prognostic value. Several children with low initial titers later developed high titers.

The high frequency of IgE antibodies to soy bean indicates that soy bean is a potent immunogen. The low clinical significance might indicate an efficient gastrointestinal barrier to soy bean allergen.

In contrast to another study (7) children with delayed reactions to cow's milk (Group II) seldom had IgE antibodies or positive skin tests to milk. The reaction type to challenge in these children was more suggestive of a type III reaction if immunological at all.

In conclusion cow's milk sensitivity remains a clinical diagnosis based on the outcome of elimination and re introduction of cow's milk in the diet. However it is now possible to discern a distinct entity of cow's milk allergy based on reaginic mechanisms in individuals predisposed to allergic disease.

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REFERENCES

1. Bachmann E D & Dees S C. Milk allergy II. Observations on incidence and symptoms of allergy to milk in allergic infants. *Pediatrics* 20: 400, 1957.
2. Bok S A, Buchley J, Holst A & May C D. Proper use of skin test with food extracts in the diagnosis of hypersensitivity to food in children. *Clin Allergy* 7: 375, 1977.
3. Brechley P E C. IgE in milk allergy. *Postgrad Med J* 51: 76 Suppl 3, 1975.
4. Ceska M & Lundkvist U. A new and simple radioimmunoassay method for determination of IgE. *Immunochimistry* 9: 103, 1977.

- 5 Dannaeus A, Johansson S G O, Foucard T & Ohman S. Clinical and immunological aspects of allergy in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 66: 31, 1977.
- 6 Foucard T. A follow up study of children with asthmatic bronchitis. I. Skin test reactions and IgE antibodies to common allergens. *Acta Paediatr Scand* 62: 633, 1973.
- 7 Fallstrom S P, Ahlstedt S & Hansson L A. Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl Immunol* 56: 97, 1978.
- 8 Gerrard J W, MacKenzie J W A, Goluboff H, Garson J Z & Manning C S. Cow's milk allergy: Prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* Suppl 234, 1973.
- 9 Goldman A S. Cow milk sensitivity: A review in Food and Immunology. *Swedish Nutrition Symp VIII* 1977, p 99.
- 10 Johansson S G O, Bennich H & Berg T. In vitro diagnosis of atopic allergy. III. Quantitative estimation of circulating IgE antibodies by the radio-allergosorbent test. *Int Arch Allergy Appl Immunol* 41: 443, 1971.
- 11 Kletter B, Gery I, Freier S, Noah Z & Davies M A. Immunoglobulin E antibodies to milk proteins. *Clin Allergy* 1: 749, 1971.
- 12 Matthews T S & Soothill J F. Complement activations after milk feeding in children with cow's milk allergy. *Lancet* II: 893, 1970.
- 13 Parish W E. Detection of reaginic and short term sensitizing anaphylactoid antibodies to milk in sera of allergic and normal persons. *Clin Allergy* 1: 369, 1971.
- 14 Peterson R D A & Good R A. Antibodies to cow's milk—their presence and significance. *Pediatrics* 31: 209, 1963.
- 15 Rothberg R M & Farr R S. Incidence and amount of antibovine serum albumin (BSA) and anti alpha lactalbumin (ALA) in serum of children and adults. *Clin Res* 10: 219, 1962.
- 16 Savilahti E. Immunochemical study of malabsorption syndrome with cow's milk intolerance. *Gut* 14: 491, 1973.
- 17 Scheinmann P, Gendrek D, Charles J & Baupé J. Value of lymphoblast transformation test in cow's milk protein intolerance. *Clin Allergy* 6: 515, 1976.
- 18 Spies J R, Stevan M A & Stein W J. The chemistry of allergens. XII. Eight new antigens generated by successive pepsin hydrolyses of bovine β lactoglobulin. *J Allergy Clin Immunol* 50: 89, 1973.
- 19 Walker Smith J. Cow's milk protein intolerance. Transient food intolerance of infancy. *Arch Dis Child* 50: 347, 1975.

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COW'S MILK ALLERGY INCIDENCE AND PATHOGENETIC ROLE OF EARLY EXPOSURE TO COW'S MILK FORMULA

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ABSTRACT Stintzing G and Zetterstrom R (Department of Paediatrics, St. Goran's Children's Hospital, Stockholm, Sweden). Cow's milk allergy incidence and pathogenetic role of early exposure to cow's milk formula. *Acta Paediatr Scand* 68: 383, 1979. A study was performed in infants under the age of 12 months born during 1974 and admitted to St. Goran's Children's Hospital with symptoms suggestive of cow's milk allergy (CMA). The aims of the study were to determine the role of early exposure to cow's milk formulas as a predisposing factor to CMA and to estimate the incidence of CMA in infancy. Twenty-five infants fulfilled the criteria for CMA. Available records were reviewed and a careful history was obtained from the mothers on two occasions. The patient group was compared with a control group. Sixteen of the 25 infants were exposed to cow's milk protein during their first week in the nursery for newborns: 6 were exposed before the end of the fourth week of life and 3 infants were apparently not exposed. All infants were breast fed 3-26 weeks before re-exposure and occurrence of symptoms. Infants with CMA were given cow's milk formulas during their first 4 weeks of life significantly more often than infants in the control group ($p < 0.01$). The incidence of CMA was approximately 1/200. The first 4 weeks after birth seem to be a particularly vulnerable period. Hence, in order to prevent CMA, infant formula should not be given—even occasionally—during this period.

KEY WORDS Cow's milk allergy, neonatal feeding

Cow's milk allergy (CMA) seems to be more prevalent in societies where breast feeding is abandoned and replaced by artificial feeding at an early age. As in all allergic disorders an episode of sensitization must precede the onset of allergic manifestations. Wernstedt reported in 1910 that infants who developed anaphylactic reactions to cow's milk were fed cow's milk shortly after birth. Recently it has been suggested that the pathogenetic mechanism is related to a transient defect in the immune apparatus in early life which is primarily related to secretory IgA of the gut (10). This leads to defective assimilation of the antigen, e.g. of cow's milk protein, which initiates the allergic process. Although it has been discussed that sensitization may even take place *in utero* (6), the first weeks after birth seem to be a particularly vulnerable period from this point of view.

The reported incidence of CMA varies widely (0.3-7%) (2, 5). There are many pos-

sible explanations for this discrepancy, e.g. lack of common clinical criteria and different feeding habits in early life.

Two brands of infant formulas are currently available in Sweden, both are based on cow's milk and have a similar composition. They are both processed by spray-drying and have an average protein content of 13.4 g/l and a casein-whey protein ratio of 40:60 (energy 2,900 kJ/l, fat 35 g/l, of which linoleic acid 6 g/l, lactose 72 g/l).

This study was undertaken to elucidate the possible pathogenetic role of early exposure to infant formulas in the pathogenesis of CMA and to estimate the incidence of CMA in infancy.

PATIENTS AND METHODS

Children under the age of 12 months admitted to St. Goran's Children's Hospital with symptoms suggestive of CMA and who were born during 1974 were investigated. The following data were obtained on admission: case history, physical examination, routine analyses of blood

- 5 Danneus A, Johansson S G O, Foucard T & Ohman S. Clinical and immunological aspects of allergy in childhood. I. Estimation of IgG, IgA, and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 66: 31, 1977.
- 6 Foucard T. A follow up study of children with asthmatic bronchitis. I. Skin test reactions and IgE antibodies to common allergens. *Acta Paediatr Scand* 67: 633, 1973.
- 7 Fallstrom S, P, Ahlstedt S & Hansson I. A. Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl Immunol* 56: 97, 1978.
- 8 Gerrard J W, Mackenzie J W A, Goluboff N, Garson J Z & Manning C S. Cow's milk allergy: Prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* Suppl. 234, 1973.
- 9 Goldman A S. Cow milk sensitivity: A review in Food and Immunology. *Swedish Nutrition Symp* VIII, 1977, p. 99.
- 10 Johansson S G O, Bennich H & Berg T. In vitro diagnosis of atopic allergy. III. Quantitative estimation of circulating IgE antibodies by the radioallergen sorbent test. *Int Arch Allergy Appl Immunol* 41: 443, 1971.
- 11 Kletter H, Gery I, Freier S, North Z & Davies M A. Immunoglobulin G antibodies to milk proteins. *Clin Allergy* 1: 249, 1971.
- 12 Matthews T S & Soothill J F. Complement activations after milk feeding in children with cow's milk allergy. *Lancet* II: 893, 1970.
- 13 Pansh W E. Detection of reaginic and short acting sensitizing anaphylactoid antibodies to milk in allergic and normal persons. *Clin Allergy* 1: 1971.
- 14 Peterson R D A & Good R A. Antibodies to cow's milk—their presence and significance. *Pediatrics* 31: 209, 1963.
- 15 Rothberg M M & Farr R S. Incidence and analysis of antiovine serum albumin (BSA) and anti-albumin (ALA) in serum of children and adults. *Clin Res* 10: 219, 1962.
- 16 Savilhti E. Immunochemical study of malabsorption syndrome with cow's milk intolerance. *Gut* 491, 1973.
- 17 Scheinmann P, Gendrek D, Charles J & Paul J. Value of lymphoblast transformation test in cow's milk protein intolerance. *Clin Allergy* 11: 515, 1974.
- 18 Spies J R, Stevan M A & Stein W J. The chemistry of allergens. VII. Eight new antigens generated by successive pepsin hydrolyses of bovine β lactoglobulin. *J Allergy Clin Immunol* 50: 82, 1973.
- 19 Walker-Smith J. Cow's milk protein intolerance: Transient food intolerance of infancy. *Arch Dis Child* 50: 347, 1975.

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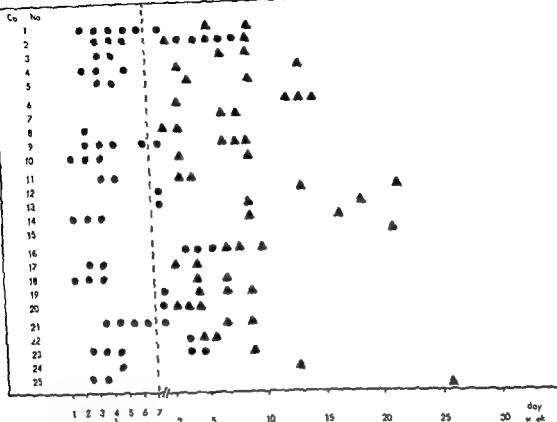


Fig. 1 Patients with CMA. ● The day or week the patient was given any infant formula without reacting to it. ▲ the week an allergic symptom was noted.

Sixteen patients were given the formula on one or more occasions on the second to the seventh day, most frequently on the fourth day. The amount of formula given on any day ranged from 10 to 300 ml. In 2 cases the patient received the formula only once. In 10 instances the mother did not know or had forgotten about this early exposure to cow's milk protein. All 25 babies were fully or at least partly breast fed at the time of discharge from the newborn nursery.

Of the remaining 9 babies, 6 were given infant formula within the first 4 weeks of life. In only 3 cases was no evidence obtained of any previous exposure to cow's milk protein. Fig. 1 also shows that one patient developed symptoms on the first re-exposure to cow's milk protein as late as the 26th week.

The symptoms are listed in Table 2. Twenty-one of the patients (84%) had gastrointestinal and 10 (40%) cutaneous symptoms. In 4 cases the latter were the only manifestations of CMA. Four patients (16%) developed respiratory symptoms. Shock was seen in 2 patients. In 23 of 25 patients no untoward reactions were observed following ingestion of the soy-based formula. The 2 infants who developed symptoms on soy formula tolerated Nutramigen® well.

The control group consisted of 52 infants, 48 were breast fed at the time of discharge from the newborn nursery and the remaining 4 infants were not. In 47 cases it was possible to contact the mothers one year after for a follow-up dietary history. The amount of formula given on any day varied from 10 to

Table 1 Patients with CMA

No of patients	Boys/girls	Age at first exposure (mean and range)	No of infants exposed in the newborn nursery (day 1-7)	Age at onset of symptoms (mean and range)	Age on admission (mean and range)
25	16/9	1½ w 2 d-4 w	16	7½ w 3-26 w	10½ w 5-26 w

including immunoglobulins skin test or intradermal test to cow's milk and a careful dietary history from each mother. Records from the newborn nursery, well baby clinics and other hospitals were reviewed for information concerning previous health status, weight gain and dietary history. Regular follow up in the out patient department consisted of a physical examination and a dietary history. Note was made of the day or week the patient was given infant formula and the week when symptoms occurred.

The criteria for CMA in this study are identical with those used by Goldman (7): 1) subsidence of symptoms after dietary elimination of cow's milk, 2) recurrence of symptoms within 48 hours after challenge with cow's milk and 3) positive reactions showing similar onset duration and clinical features to three such challenges. Thus each patient was challenged one or two times with full cow's milk and on one occasion with the same formula that induced symptoms prior to admission. The patient was always under professional supervision when challenged. The challenge started with 2-5 ml followed at 2 hour intervals with 5, 10, 20, 40 ml etc. until the ordinary amount for the patient's feed was reached or stopped if some reaction was registered.

On admission and after challenge the patient was given a soy based formula ProSobee[®], Sobee[®] or secondarily a casein hydrolysate Nutramigen[®] unless he could be fully breast fed. Symptoms were listed under three main headings: 1) gastrointestinal e.g. vomiting and diarrhoea, 2) cutaneous e.g. rash, urticaria and eczema and 3) respiratory e.g. rhinitis, asthmatic symptoms. Shock was noted separately.

The control group consisted of the last baby born on a Monday and the first baby born on a Thursday in one of the nurseries for the newborn during the first half year of 1974. Fifty two babies, 28 boys and 24 girls, were included. Records from the newborn nursery, well baby clinics and other hospitals were reviewed. The mother was interviewed regarding the dietary and health history of her baby when he/she had reached an age of about 12 months. In a few cases the mother and her baby were seen in the out patient department.

RESULTS

Twenty five infants fulfilled the criteria for CMA. They were all born at term with appropriate weight for gestational age. Prior to the onset of allergic symptoms all infants

were healthy. Routine blood analyses including serum immunoglobulins were normal.

Sixteen of the infants were boys and 9 were girls. In 16 cases the patient received a cow's milk formula in the newborn nursery. The mean age at onset of symptoms was 7½ weeks and on admission 10½ weeks (see Table 1).

Fig. 1 shows the day or the week the baby received formula with or without reacting to it.

Table 2 Symptoms of patients with CMA

Case no	Symptoms		
	Gastro-intestinal	Cutaneous	Respiratory
1	+		
2		+	+
3	+		
4	+	+	
5	+	+	
6		+	
7	+		+
8	+		
9	+		
10	+		
11	+		+
12	+	+	
13	+		
14	+		
15	+		
16	+	+	
17	+		
18	+	+	+
19	+	+	
20	+		
21	+		
22		+	
23	+		
24	+		
25		+	
21		10	4
84		40	16

Symptoms of shock

infants who may have had this symptom as the only manifestation of CMA. It is also possible that a number of infants have mild symptoms of CMA which subside spontaneously. Since no such infants have ever come to this hospital none are included in this study.

The fact remains that the occasional and often forgotten exposure to cow's milk protein seems to play an important role in the pathogenesis of CMA as recently suggested by Buisseret (1). Altered composition of infant formulas and changed feeding habits of newborns and small infants may play an important role as well. The most important prophylactic measure must be encouragement of breast feeding from the first day of life and strict avoidance of unnecessary and occasional bottle feeding with cow's milk based formulas at least during the first month of life.

REFERENCES

- 1 Buisseret M D Common manifestations of cow's milk allergy in children *Lancet* i 304 1978
- 2 Collins Williams C The incidence of milk allergy in paediatric practice *J Pediatr* 48 39 1966
- 3 Danneberg A Johansson S G O & Foucard T Clinical and immunological aspects of food allergy in childhood II *Acta Paediatr Scand* 67 497 1978
- 4 Gerrard J W Lubos M C Hardy L W Holm lund B A & Webster D Milk allergy clinical pic

- ture and familial incidence *Can Med Assoc J* 97 780 1967
- 5 Gerrard J W Mackenzie J W A Goluboff N Garson J Z & Maningas C S Cow's milk allergy Prevalence and manifestations in an unselected series of newborns *Acta Paediatr Scand Suppl* 234 1973
- 6 Glaser J & Johnstone D C Prophylaxis of allergic disease in the newborn *JAMA* 153 670 1953
- 7 Goldman A S Anderson D W Sellers W A Saperstein S Kniker W T & Halpern S R Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics* 39 425 1963
- 8 Goldman A S Sensitivity to cow's milk In L Hambræus L Å Hanson & H McFarlane (eds) *Food and Immunology* Almquist & Wiksell Stockholm 1977 p 99
- 9 Savilahti E Immunochemical study of the malabsorption syndrome with cow's milk protein intolerance *Gut* 14 491 1973
- 10 Sothill J F Immunodeficiency allergy and infant feeding In L Hambræus L Å Hanson & H McFarlane (eds) *Food and Immunology* Almquist & Wiksell Stockholm 1977 p 88
- 11 Wernstedt W Zur Frage der Kuhmilchdiagnose im Säuglingsalter *Monatsschr Kinderheilkd* 9 345 1910

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300 ml. In 8 of 19 cases the mother was unaware of or had forgotten that her baby had been given formula in the newborn period. Another 6 patients were either occasionally or regularly given some infant formula during the first 4 weeks of life. Except for one case that could not be followed up, no baby in the control group had shown symptoms attributable to CMA during the first year of life.

In comparison with infants in the control group, the patients with CMA were given infant formula significantly more often during their week-long stay in the newborn nursery, as well as during their first 4 weeks of life (χ^2 analysis $p < 0.05$ for first week and $p < 0.01$ for first 4 weeks of life).

Five of the patients were cared for in the same newborn nursery during the first half of 1974 as the babies in the control group. During this period, a total of 1024 babies were admitted to this nursery, which gives an incidence of 0.49% for CMA for this nursery. Some 4311 babies were born in the entire year from which all patients are admitted to this children's hospital. Since the total number of patients with CMA was 25, the calculated incidence for the year is 0.58%. This means that approximately one baby in 200 will develop CMA during the first year of life.

DISCUSSION

In this study we found that of 25 infants who developed CMA, 22 received cow's milk formula on at least one occasion during the first 4 weeks of life and 16 of them received some formula even during the first week after birth. It is also of interest that all babies were at least partly breast-fed for some time before the onset of symptoms of CMA. Our observations thus accord with those of Wernstedt (11) which were reported in 1910. Collins Williams (2) likewise concludes that it is probably more deleterious to give infant formulas just occasionally rather than regularly in early life. In Sweden, newborns usually remain in the newborn nursery during the first week of life. It

is therefore noteworthy that many mothers did not know or had forgotten that their baby had been given infant formula during this period. In the control group we also found that it was a fairly common practice to give infant formula occasionally in the newborn nursery. This apparently occurs because the staff is uncertain whether the mothers will have enough breast milk and because they want to induce rapid weight gain. This ambition may represent an extension of the practice of early feeding of small for gestational age babies to full term healthy newborns. However, it is not possible to exclude exposure to cow's milk protein even if the baby was never given formula deliberately. Since foods ingested by the mother can cause allergic reactions in breast-fed babies (4), this might explain how the 3 cases who had no known previous direct exposure to cow's milk protein developed CMA. Sensitization *in utero* might be another possibility (6).

All of the principal proteins in cow's milk have been incriminated in the pathogenesis of CMA (7) and complex immunologic mechanisms (9) including IgE antibodies (3) are involved in the allergic reaction. The reduced content of casein in relation to whey proteins is the case with so-called humanized infant formulas currently in use and the processing itself may render formulas more allergenic than cow's milk.

It is generally believed that the incidence of CMA has increased in recent years. Our incidence of about 0.5% is low in comparison to that reported by Gerrard et al. (5) as 7.5% but using Goldman and co-workers' criteria it is similar to that suggested by the same author in 1977 for the United States, viz. 0.5–1.0%. However, figures for incidence are somewhat difficult to compare because of various factors related to selection. Another reason for the wide range of the reported incidence is that different criteria are used for the diagnosis of CMA in different studies. Due to difficulties in evaluating such ill-defined symptoms as colic we have been forced to exclude

infants who may have had this symptom as the only manifestation of CMA. It is also possible that a number of infants have mild symptoms of CMA which subside spontaneously. Since no such infants have ever come to this hospital none are included in this study.

The fact remains that the occasional and often forgotten exposure to cow's milk protein seems to play an important role in the pathogenesis of CMA as recently suggested by Buisseret (1). Altered composition of infant formulas and changed feeding habits of newborns and small infants may play an important role as well. The most important prophylactic measure must be encouragement of breast feeding from the first day of life and strict avoidance of unnecessary and occasional bottle feeding with cow's milk based formulas at least during the first month of life.

REFERENCES

- 1 Buisseret P D. Common manifestations of cow's milk allergy in children. *Lancet* **i** 304 1978.
- 2 Collins Williams C. The incidence of milk allergy in pediatric practice. *J Pediatr* **48** 39 1956.
- 3 Dannaeus A, Johansson S G O & Foucard T. Clinical and immunological aspects of food allergy in childhood. II. *Acta Paediatr Scand* **67** 497 1978.
- 4 Gerrard J W, Lubos M C, Hardy L W, Holmlund B A & Webster D. Milk allergy: clinical pic-

ture and familial incidence. *Can Med Assoc J* **97** 780 1967.

- 5 Gerrard J W, Mackenzie J W A, Goluboff N, Garson J III & Marungas C S. Cow's milk allergy: Prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* Suppl **234** 1973.
- 6 Glaser J & Johnstone D C. Prophylaxis of allergic disease in the newborn. *JAMA* **153** 670 1953.
- 7 Goldman A S, Anderson D W, Sellers W A, Saperstein S, Kniker W T & Halpern M R. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* **37** 425 1963.
- 8 Goldman A S. Sensitivity to cow's milk. In L. Hambræus, L. Å. Hanson & H. McFarlane (eds). *Food and Immunology*. Almqvist & Wiksell, Stockholm 1977, p. 99.
- 9 Savilahti E. Immunochemical study of the malabsorption syndrome with cow's milk protein intolerance. *Gut* **14** 491 1973.
- 10 Soothill J F. Immunodeficiency, allergy and infant feeding. In L. Hambræus, L. Å. Hanson & H. McFarlane (eds). *Food and Immunology*. Almqvist & Wiksell, Stockholm 1977, p. 111.
- 11 Wernstedt W. Zur Frage der Kuhmilchdysynkrasie im Säuglingsalter. *Monatsschr Kind rheilkd* **9** 345 1910.

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HUMAN COLOSTRAL AND BREAST MILK CELLS

A Light and Electron Microscopic Study

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ABSTRACT Ho F C S, Wong R L C and Lawton J W M (Departments of Pathology and Obstetrics and Gynecology, University of Hong Kong). Human colostrum and breast milk cells: a light and electron microscopic study. *Acta Paediatr Scand* 68: 389-396, 1979. — Colostrum and breast milk samples were obtained from 74 women, 18 of whom gave sequential samples. The mean total leukocyte count in colostrum was 3 190 cells/mm³. Proportions of macrophages, polymorphs and lymphocytes varied widely; macrophages usually predominated. Serial sampling showed (1) a small fall in total counts through delivery, (2) a fall in total counts and the proportion of PMNs at the onset of lactation, (3) after 1 to 2 weeks of lactation the appearance of cytoplasmic fragments together with epithelial cells which later constituted the main cell type. It was estimated that the total number of leukocytes available in the neonate remained approximately constant during the first 2 weeks of lactation and fell thereafter. Functionally, morphologically and histochemically macrophages in colostrum and breast milk resembled macrophages elsewhere. Their ultrastructure was characterised by filiform surface projections, numerous endocytic vacuoles and lipid droplets in the cytoplasm.

KEY WORDS Colostrum, breast milk, leukocytes, macrophages, lactation, electron microscopy.

The presence of leukocytes in human colostrum and breast milk has long been recognized but has received scant attention. These cells comprise macrophages, polymorphonuclear leukocytes (PMNs) and lymphocytes (11-12, 20). Results of recent studies have indicated that they may play an important role in transferring specific and non-specific host resistance factors to the neonate. The mononuclear cells in colostrum may contribute specifically by synthesizing IgA antibodies (14), particularly against enteric bacteria (1) and non-specifically by synthesizing complement (14), lysozyme (11) and interferon (5, 10). Lymphocytes in colostrum and breast milk respond to mitogens, recall antigens and allogeneic cells (4, 17, 20) and there is evidence that a component of breast secretions can passively transfer cell-mediated immunity to the infant (13), although this may not be long-lasting (15). The macrophages and PMNs may protect the

neonate by their capacity to ingest and to kill microorganisms (18); they can ingest *C. albicans* and *E. coli* as avidly as blood leukocytes but their killing capacity is much less (7). There is also evidence that breast milk macrophages may act as a vehicle of immunoglobulin transport (19).

In early postpartum colostrum the total leukocyte counts are comparable to those in peripheral blood counts ranging from 500 to 8000/mm³; have been found macrophages making up 30 to 60% of the cells. As lactation is established the cell concentration drops while the proportion of macrophages rises (11).

In this study of a predominantly Chinese population we set out to extend this knowledge by doing serial studies of cell counts in colostrum and breast milk through parturition and established lactation and to further characterize the cells by cytochemical stains and

by electron microscopy. This formed part of a wider study of the function of these cells and their possible significance to the neonate.

MATERIALS AND METHODS

Colostrum and breast milk samples from 74 women (67 Chinese, 6 Caucasian, 1 Indian) were obtained by manual expression into sterile plastic Universal bottles. Fifty-six donors gave one sample only. Sequential samples were donated by 18 women, 3 of them up to 6 months of lactation. The data referred to below as data from random samples was collated from all individual samples and appropriately timed sequential samples. In addition all data derived from sequential samples was tabulated separately (see all samples).

Preparation of cells

Colostrum diluted 1/2 in Hanks Balanced Salt Solution (HBSS) and undiluted breast milk was spun at 100×g for 10 min. The cells were washed 2× in HBSS prior to suspension in medium RPMI 1640 containing HEPES/bicarbonate buffer, penicillin and streptomycin and 20% heat inactivated human AB serum. Fifty thousand cells in 1 to 2 ml were spun onto a circular glass coverslip (18 mm diam) at 100×g for 5 min. The coverslip was air dried prior to Wright's staining for differential counts.

Cytochemistry

Cells prepared by cytocentrifugation were stained for non-specific esterase (using α -naphthyl acetate as substrate), chloroacetate esterase (22), acid phosphatase and myeloperoxidase (MPO) and with Oil Red O.

Phagocytosis

Cells suspended in LGH were exposed to a suspension of polystyrene particles (0.05% 0.8 μ m diam) at room temperature for 30 min. To assess phagocytosis of bacteria, cells were suspended in HBSS containing 20% fresh AB serum as a source of opsonins. Heat-killed *S. aureus* or *E. coli* were added (approximately 10 organisms per cell) and the mixture was incubated at 37°C for 30 min in a shaking water bath.

Electron microscopy

Aliquots of approximately 1×10^6 washed cells were fixed in suspension in 2.5% glutaraldehyde for 4–6 hours, post-fixed in 1% phosphate buffered osmium tetroxide and to the centrifuged pellet were added a few drops of 2% agarose gel at 60°C. The cells in agarose were pipetted onto a dental wax plate and when set the gel was cut into small cubes. These were dehydrated in increasing concentrations of ethanol and transferred through propylene oxide to 1-pon 812. Thin sections of selected blocks were stained with uranyl acetate and lead nitrate and examined in a Philips EM300 electron microscope. In 3 of the 8 samples studied, separate aliquots of cells were first exposed to *S. aureus* then processed as described above.

RESULTS

Cell morphology

Mononuclear phagocytes (MPs) were arbitrarily classified as small macrophages < 20 μ m in diameter and large macrophages > 20–40 μ m in diameter (including the corpuscles of Donné) but the cells display a continuum of appearances from the smallest (with scanty cytoplasm resembling lymphocytes) to the largest with copious vacuolated cytoplasm (Fig. 1). Occasional cells closely resembled blood monocytes. Large macrophages often had ingested cellular material in the cytoplasm and were sometimes multinucleate.

Both small and large macrophages were shown to be actively phagocytic for *S. aureus*, *E. coli* and polystyrene particles.

Epithelial cells were typically 15 to 20 μ m in diameter, had deeply basophilic cytoplasm and a tendency to occur in clumps of 2 to 4 cells (Fig. 2a). They were not phagocytic. Cytoplasmic fragments showed staining characteristics similar to the epithelial cell cytoplasm (Fig. 2b).

Polymorphs were mostly neutrophilic, although occasional eosinophil was seen. Often large cytoplasmic vacuoles were present, pushing the nucleus to the periphery. PMNs were less avidly phagocytic for bacteria and polystyrene particles than were the macrophages.

Lymphocytes were morphologically unremarkable.

Cell counts on random samples

Random sampling showed little change in absolute counts or in proportions of leukocyte types from the prepartum to the immediate post partum period (Table 1). As lactation became established the total count dropped steeply to a mean of about 400/mm³ at the end of the first week, while the mean proportion of macrophages rose from 49% to 72%. Equally striking was the reciprocal fall in numbers of polymorphs as the milk flow increased. The mean percentage of lymphocytes was fairly

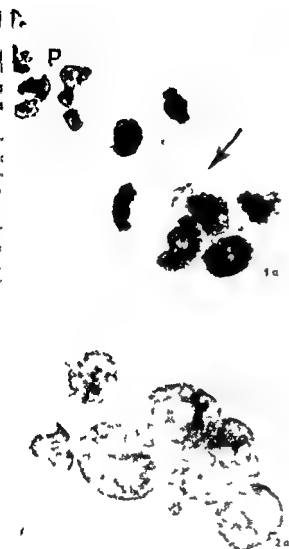


Fig. 1 (a) Small macrophages (arrowed) and PMNs (P) in prepartum colostrum. The small macrophages have moderately basophilic finely vacuolated cytoplasm and occasional azurophilic granules. The nucleus is round, oval, reniform or irregular and eccentrically placed with moderately condensed chromatin containing 1 to 3 small nucleoli.

(b) A large macrophage in prepartum colostrum. The cytoplasmic vacuoles are numerous and prominent giving the cell its characteristic foamy appearance. Foamy macrophages not uncommonly have a centrally placed nucleus (see Fig. 2A) (Wright's stain $\times 1750$).



Fig. 2 (a) A clump of 4 epithelial cells and a foamy macrophage in 3 week breast milk. The epithelial cells have deeply basophilic cytoplasm, a round, eccentrically placed nucleus with smooth or finely fenestrated chromatin. Clear secretory vacuoles in the cytoplasm vary greatly in number and size.

(b) Epithelial cells in 3 week breast milk together with cytoplasmic fragments. Many of these fragments contained a single large vacuole (Wright's stain $\times 1250$).

Table 1 Cell counts on random samples of colostrum and early breast milk

	Total count mean cells per mm ³	Macrophages		Polymorphonuclear leukocytes		Lymphocytes	
		Mean %	Mean cells per mm ³	Mean %	Mean cells per mm ³	Mean %	Mean cells per mm ³
<i>Prepartum</i>							
3-1 weeks (n=11)	3 370 (950-11 250)	40.5 (11-68)	1 200 (760-1 920)	52.7 (13-87)	2 240 (120-9 790)	5.7 (1-19)	170 (40-5 000)
7-0 days (n=28)	1 570 (750-9 400)	52.6 (9-89)	1 700 (90-6 940)	38.6 (2-83)	1 500 (80-5 360)	8.5 (0-13)	189 (0-560)
<i>Postpartum</i>							
0-2 days (n=16)	2 810 (100-14 000)	49.1 (8-84)	1 220 (50-4 760)	44.3 (9-69)	1 430 (55-5 040)	6.6 (2-23)	180 (2-1 800)
3-4 days (n=14)	1 140 (65-6 000)	48.6 (8-94)	390 (40-2 460)	44.9 (3-97)	701 (20-5 640)	5.4 (0-16)	47 (1-09)
5-8 days (n=12)	439 (53-1 970)	72.2 (46-97)	315 (14-1 670)	20.0 (5-53)	96 (3-419)	5.3 (1-12)	27 (1-194)

Ranges of values are given in parentheses

constant in the first week postpartum (5.3% to 6.6%)

By the second week of lactation the absolute numbers of all cell types had dropped markedly, probably due to a dilution effect.

Counts on serial samples

Serial sampling revealed trends which were not apparent by random sampling (Table 2). From pre- to postpartum colostrum there was a small drop in mean total cell count, a fall in

Table 2 Cell counts on serial samples of colostrum and milk*

	Total count mean cells per mm ³	Macrophages		Polymorphonuclear leukocytes		Lymphocytes		Epithelial cells	
		Mean %	Mean cells per mm ³	Mean %	Mean cells per mm ³	Mean %	Mean cells per mm ³	Mean %	Mean cells per mm ³
<i>Pre partum</i>									
7-0 days (n=7)	3 430 (1 100-7 800) ^a	66 (37-93)	2 140 (350-6 940)	21 (2-41)	560 (10-900)	11 (2-36)	740 (70-390)		
<i>Post partum</i>									
0-4 days (n=14)	2 840 (300-14 000)	48 (24-87)	1 300 (50-4 760)	42 (11-67)	1 350 (60-7 470)	7.3 (1-17)	207 (7-1 870)	<1	-
5-8 days (n=12)	439 (50-1 970)	77 (46-92)	315 (14-1 620)	20 (5-53)	96 (3-470)	5.3 (1-12)	77 (1-190)	75 (0-5)	<1
1-2 weeks (n=7)	69 (4-110)	75 (61-97)	57 (7-130)	5.6 (2-23)	4 (1-7)	2.9 (0-13)	1 (0-1)	5.6 (0-23)	4 (0-9)
2-4 weeks (n=9)	51 (15-167)	44 (2-88)	22 (1-84)	16 (1-89)	8 (1-37)	2.2 (0-4)	1 (0-4)	76 (7-80)	73 (1-80)
1-2 months (n=5)	17 (5-30)	24 (2-38)	4 (1-11)	10 (1-25)	3 (1-8)	7.2 (0-27)	1 (0-4)	56 (53-96)	8 (5-15)
2-4 months (n=6)	16 (3-60)	11 (0-47)	5 (0-31)	18 (1-67)	2 (0-8)	7.5 (0-14)	1 (0-9)	71 (28-94)	9 (3-70)
4-6 months (n=5)	10 (3-77)	2.7 (0-6)	<1 (0-1)	4.6 (0-18)	<1 -	0.9 (0-8)	<1 -	84 (58-99)	9 (7-6)

Samples donated by a total of 18 individuals

* Range of values in parentheses



Fig 3 Epithelial cell in day 4 breast milk. The cell outline is smooth and the nucleus large and ovoid. There are numerous free ribosomes and some short profiles of rough endoplasmic reticulum. One fat droplet (d) and several vesicles are seen at one pole of the cell. Electron micrograph ($\times 5600$).

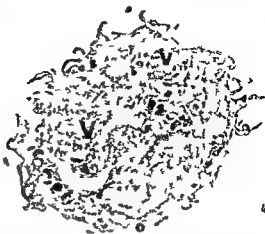


Fig 4 Macrophage in prepartum colostrum with characteristic surface projections. The flattened nucleus shows moderate peripheral condensation of chromatin. Two large vacuoles (V) containing cellular debris are seen. Electron micrograph ($\times 5600$).

macrophage and lymphocyte percentages and a rise in the number of PMNs.

During the second week of lactation epithelial cells were found in small numbers (0–23%) accompanied by numerous cell fragments probably derived from the process of apocrine secretion. While the absolute numbers of leukocytes continued to fall the epithelial cells reached the highest concentration of 23 cells/mm³ late in the first month and thereafter became the predominant cell type.

Cytochemistry

The macrophages stained strongly for non-specific esterase (fluoride inhibited) and acid phosphatase. They were usually negative for MPO and chloro acetate esterase. The staining pattern with Oil Red O indicated that the abundant cytoplasmic vacuoles in macrophages and PMNs were lipid containing.

Colostrum PMNs stained weakly for both chloro acetate esterase and MPO in contrast to the strong reactions given by blood PMNs (22). The epithelial cells and cell fragments stained for non-specific esterase and acid phosphatase and contained lipid droplets.

Electron microscopy

Of the range of cell types seen most numerous were MPs of varying morphology and PMNs but there were occasional lymphocytes and epithelial cells (Fig. 3) as well as fragments of epithelial cell cytoplasm.

Cells of the MP series were identified by an indented or irregularly shaped nucleus, their relatively abundant cytoplasm, large veil-like and smaller filiform projections from their surface membrane and the presence of endocytic vesicles containing material of varied nature. Some vacuoles contained scanty flocculent material whereas others had a moderately electron dense homogeneous content consistent with lipid droplets. Phagocytosed cell debris and phagosomes with dense material (residual bodies) were also seen (Fig. 4). The cells containing numerous lipid droplets of uniform size (Fig. 5) are probably the foam cells (Corpuscles of Donne) seen by light microscopy. Transitional forms between these cells and typical MPs and phagocytosis of *S. aureus* by them (Fig. 6) indicate their macrophage origin. Other organelles were clearly seen only in the cells with fewer lipid droplets; these in

b



5



6

Fig. 5. Macrophage in prepartum colostrum containing numerous lipid droplets, some arrowed. These cells are considered the equivalent of the foamy macrophages seen by light microscopy. Electron micrograph ($\times 5600$).

Fig. 6. Macrophage in prepartum colostrum after incubation with heat-inactivated *S. aureus*. The ingested cocci are arrowed. This cell resembles that in Fig. 5 but has larger veil-like cytoplasmic projections, sometimes folded back to enclose small vesicles. Electron micrograph ($\times 8100$).



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Fig. 7. A group of neutrophils in day 4 breast milk. These cells show a heavy peripheral condensation of chromatin in their segmented nuclei. Endocytic vesicles with heterogeneous contents are seen. Electron micrograph ($\times 4600$).

cluded small scattered mitochondria, a usually well developed Golgi apparatus, strands of rough endoplasmic reticulum near the periphery of the cell and many free ribosomes. Where these cells were in contact with cytoplasmic fragments, fingerlike projections and deep invaginations of the surface membrane were abundant. In many fields several cells surrounded and enclosed cellular debris or large lipid globules.

PMNs were characterized by their multilobed nucleus with heavy peripheral condensation of chromatin (Fig. 7). Although they also contained a range of inclusions similar to the

MPs, none of them showed signs of transformation into foam cells. Specific granules were preserved in only a few cells and degenerative changes were more advanced and more evident in PMNs than in MPs. The variations observed in the preservation of fine structure of these and other cells within the same sample may be related not only to differences in their natural lifespan but also to the duration of their immersion in the rather special environment of the colostrum.

Lymphocytes had a high nuclear:cytoplasmic ratio, a few short uniformly distributed surface projections, abundant cytoplasmic

polyribosomes and a few strands of rough endoplasmic reticulum. Mitochondria were usually grouped on one side of the nucleus but we did not observe further evidence of polarity in these cells as reported by Smith et al. (21). These investigators allowed the cells to settle on a cover glass to facilitate interaction whereas in our work the cells were fixed in suspension. However in those rare instances where lymphocytes were found in close proximity to macrophages there was some interdigitation of their cytoplasmic processes.

DISCUSSION

The mean total leukocyte counts and differential counts in colostrum and early breast milk in our study are in close agreement with similar observations by other workers (11, 12, 20) but we found a wider range in total counts.

The marked fall in the proportion of PMNs and a reciprocal rise in the proportion of macrophages with onset of lactation may explain the observation of Smith & Goldman (20) that suckling is associated with a lower proportion of PMNs in colostrum. Over the first 2 weeks mean total counts fall about 50-fold while the volume of breast secretion increases from a few ml/day to about 500 ml/day. Hence the total number of leukocytes ingested by the neonate remains approximately constant during this period. After 2 weeks however total cell counts continue to fall while milk volume increases relatively little to about 750 ml/day (8).

The corpuscles of Donne (colostrum corpuscles) are now usually classified together with the other large lipid laden foamy macrophages of colostrum and early breast milk. These cells and the smaller macrophages share the characteristics of MPs found in the tissues or other body fluids: they are glass adherent, show amoeboid movement and are actively phagocytic for fungi, bacteria, colloidal particles and red cells (9, 11, 20). In tissue culture they form epithelioid and multinucleate forms and remain avidly phagocytic for poly-

styrene particles and staphylococci (unpublished observations). We have shown their ultrastructure to be similar to that of macrophages found elsewhere (2, 3) and they stain strongly for lysosomal enzymes. On the basis of observations of human mammary gland explants in culture Papanicolaou & Maddi (16) claimed that the foamy cells of breast secretions developed from epithelial cells. On the other hand their morphological, functional and histochemical characteristics suggest that they derive from cells belonging to the system of mononuclear phagocytes originating in the bone marrow and entering the breast tissues by way of the blood monocyte.

Colostrum macrophages bear a considerable morphologic resemblance to lung alveolar macrophages as described by Cline (3) and like the lipid laden alveolar macrophages in pulmonary alveolar proteinosis (6) they have a low microbicidal capacity (7). While their microbicidal capacity appears low, their phagocytic capacity is comparable to that of blood leukocytes (7) and this ability to sequester pathogens may also contribute to the protection of the breast fed neonate.

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REFERENCES

1. Ahlstedt S, Carlsson B, Hanson L Å & Goldblum R M. Antibody production by human colostrum cells. I. Immunoglobulin class specificity and quantity. *Scand J Immunol* 4: 535, 1975.
2. Carr I. *The macrophage: A review of its structure and function*. Academic Press, London, 1973.
3. Cline M J. *The white cell*. Harvard University Press, Cambridge, Massachusetts, 1975, p. 459.
4. Diaz Jouanen H & Williams H C T and H. Lymphocytes in human colostrum. *Clin Immunol Immunopathol* 3: 748, 1974.
5. Emodi G & Just M. Interferon production by lymphocytes in human milk. *Scand J Immunol* 3: 157, 1974.
6. Golde D W, Termito M, Finley T N & Cline M J. Defective lung macrophages in pulmonary alveolar proteinosis. *Ann Intern Med* 85: 304, 1976.

B



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Fig 5 Macrophage in prepartum colostrum contains numerous lipid droplets, some arrowed. These cells are considered the equivalent of the foamy macrophages seen by light microscopy. Electron micrograph ($\times 5600$)

Fig 6 Macrophage in prepartum colostrum after incubation with heat inactivated *S. aureus*. The ingested cocci are arrowed. This cell resembles that in Fig 5 but has larger veil-like cytoplasmic projections, sometimes folded back to enclose small vesicles. Electron micrograph ($\times 8100$)

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Lymphocytes had a high nuclear:cytoplasmic ratio, a few short uniformly distributed surface projections, abundant endocytic

INCIDENCE OF COELIAC DISEASE AND TRANSIENT GLUTEN INTOLERANCE IN CHILDREN IN A SWEDISH URBAN COMMUNITY

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ABSTRACT Berg N O and Lindberg T (Department of Pathology University Hospital Lund and Department of Paediatrics Malmö General Hospital Malmö University of Lund Sweden) Incidence of coeliac disease and of transient gluten intolerance in children in a Swedish urban community *Acta Paediatr Scand* 68 397 1979.—The incidence of coeliac disease in children in the city of Malmö South Sweden was 1/982 during 1966 to 1975. The diagnostic criteria were: flat intestinal mucosa on gluten-containing diet, free of symptoms and improvement in mucosal morphology on gluten-free diet and morphological and/or evident clinical relapse (three times) on gluten challenge. 6 (12%) of 49 children with initially a flat mucosa still had a normal mucosa on a gluten-containing diet for two years or longer having so-called transient gluten intolerance.

KEY WORDS Coeliac disease, gluten intolerance, intestinal biopsy.

The reported incidence of coeliac disease varies considerably (4, 5, 6, 8, 13, 15, 19, 22). This can be due to a variation in the incidence from one area to another, also to the use of different criteria for the diagnosis. Two reports are available from Sweden. They gave an incidence of 1/6500 (1950–1962) (4) and 1/3700 (1952–1963) (8). These figures are based on retrospective studies, the diagnosis being mainly determined on clinical findings.

This work reports the incidence of coeliac disease in children in a Swedish urban community during a ten year period (1966–1975). The criteria for the diagnosis were (9): a flat or nearly flat mucosa in biopsy from duodeno-jejunal flexure on a gluten-containing diet, disappearance of symptoms and a definite restitution of mucosal architecture on a gluten-free diet, a deterioration of the morphology or a definite clinical relapse (repeated three times) on the reintroduction of gluten into the diet. The frequency of so-called transient gluten intolerance (normal mucosa on gluten-containing diet for at least two years) (9) is also

PATIENTS

Malmö, a city in South Sweden with about 160 000 inhabitants (including 7% immigrants) has only one children's hospital. Children born and living in Malmö between 1 January 1966 and 31 December 1975 were included in the study. 33 405 infants were born alive in this period. The patients ($n=49$) during 1967–1976 were admitted to the hospital from the outpatient clinic and from children's welfare clinics attended by physicians from the children's hospital and by practitioner paediatricians. Table 1 lists the main symptoms and signs.

METHODS

Small intestinal biopsy was taken by a hydraulic capsule at the duodeno-jejunal flexure under fluoroscopic control (17). On average, two specimens were taken from this region on each occasion. The biopsy specimen was oriented on a mul pore filter and fixed in 4% formal solution with short after fixation in Bouin's solution. After examination and photography in a dissecting microscope, the biopsy was serially cut into 5–6 µm sections. Alternative slides were stained with haematoxylin and erythrosin with van Gieson stain and with periodic acid-Schiff according to McManus. The best oriented central cores of the specimens were used for assessment. They were classified according to Alexander (1) as follows:

normal mucosa villous (finger and leaves) (Grade I), slightly damaged mucosa ridged (Grade II), moderately damaged mucosa convoluted (Grade III), severely damaged mucosa flat mosaic (Grade IV).

- 7 Ho P C & Lawton J W M Human colostrum cells phagocytosis and killing of *E. coli* and *C. albicans* *J Pediatr* in press
- 8 Jelliffe E B P Maternal nutrition and lactation. In *Breastfeeding and the mother*. Ciba Foundation Symposium 45 North Holland Amsterdam 1976 p 119
- 9 Lascelles A K, Gurner B W & Coombs R R Some properties of human colostrum cells. *Aust J Exp Biol Med Sci* 47: 349, 1969
- 10 Lawton J W M & Shortridge A I Protective factors in human breast milk and colostrum. *Lancet* i 253, 1977
- 11 McClelland B B I personal communication
- 12 Mohr J A, Leu R & Mabry W Colostral leukocytes. *J Surg Oncol* 7: 163, 1970
- 13 Mohr J A The possible induction and/or acquisition of cellular hypersensitivity associated with ingestion of colostrum. *J Pediatr* 83: 1067, 1973
- 14 Munillo G J & Goldman A S The cells of human colostrum. II. Synthesis of IgA and β lc. *Pediatr Res* 4: 71, 1970
- 15 Ogra S S, Weintraub D & Ogra P I Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and subcellular components in the gastrointestinal tract of the newborn. *J Immunol* 119: 245, 1977
- 16 Papanicolaou G N & Muddi F V Further observations on the behaviour of human endometrial cells in tissue culture. *Am J Obstet Gynecol* 78: 156, 1959
- 17 Parmely M J, Beer A E & Billingham R E In vitro studies on the T lymphocyte population of human milk. *J Exp Med* 144: 358, 1976
- 18 Pitt J, Barlow B & Heird W C Protection against experimental necrotizing enterocolitis by maternal milk. I. Role of milk leukocytes. *Pediatr Res* 11: 906, 1977
- 19 Pittard W H, Polmar S H & Fanaroff A A The breast milk macrophage: a potential vehicle for immunoglobulin transport. *J Reticuloendotel Syst* 33: 597, 1977
- 20 Smith C W & Goldman A S The cells of human colostrum. I. In vitro studies of morphology and functions. *Pediatr Res* 2: 103, 1968
- 21 Smith C W, Goldman A S & Yates R D Interactions of lymphocytes and macrophages from human colostrum: electron microscopic studies of interacting lymphocyte. *Exp Cell Res* 69: 409, 1971
- 22 Yam I T, Li C Y & Crosby W H Cytochemical identification of monocytes and granulocytes. *Am J Clin Pathol* 55: 281, 1971

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Table 2 *Incidence of coeliac disease 1966-1975 in Malmö*

	No of live births	No of coeliac children
1966	3 815	5
1967	3 760	3
1968	3 516	
1969	3 95	4
1970	3 416	8
1971	3 563	8
1972	3 80	4
1973	3 035	7
1974	2 919	7
1975	2 806	4
Total	33 403	34 (1.98%)

23) Thus the true incidence of coeliac disease is difficult to determine. Early studies (1950 to 1963) report an incidence of 1/8000 (England and Wales (6)), 1/4000 (Scotland (6)) between 1/2000 and 1/6000 (London (5)), 1/6500 (Sweden (4)) and 1/3700 (Sweden (8)). This contrasts with recent studies with reported incidence of 1/597 (west of Ireland (19)), 1/890 (Switzerland (22)), 1/1100 (Glasgow (15)) and 1/1850 (west of Scotland (13)). The found incidence in Malmö of 1/1000 in a well defined population agrees with these latter figures. The incidence in London (5) in Switzerland (22) and in the west of Scotland (13) was estimated with the known incidence of pyloric stenosis and cystic fibrosis as a comparison. Had this method been used in this study the calculated incidence in Malmö would be between 1/400 and 1/660. The difference in incidences between the early (1950 to 1963) and the recent studies is probably due to the diagnosis being proved by routine intestinal biopsies in the later ones. Even slight forms of the disease are sent to the hospital and diagnosed by intestinal biopsy. Supporting this are the facts that 70% of the children in Malmö were diagnosed at an age of less than one year and that the frequency of the disease has increased three times since 1967 when intestinal biopsy was introduced in the routine clinical work and performed because of clinical grounds (3).

There is a strong association between coeliac

disease and the leucocyte antigen HLA B8. 60-90% of the patients are HLA B8 positive against 16-35% of the control population (24). It is of interest to note that Ireland which has the highest reported incidence of coeliac disease also has the highest HLA B8 frequency (35%) in the population (18) whereas it is 26% in the population of South Sweden (7).

Six (12%) of the 49 children with a severely damaged mucosa at the first biopsy on a gluten containing diet who became free of symptoms on a gluten free diet did not have a permanent gluten intolerance (9). The existence of this so called transient gluten intolerance is well known (10, 11, 20, 25, 26). Packer et al (21) suggest that a normal biopsy after 3 to 4 months of gluten challenge excludes a diagnosis of coeliac disease but recommend as does the European Society for Pediatric Gastroenterology and Nutrition (9) a further biopsy after two years. Accordingly it is possible that the three children with a normal mucosa after 5, 9, 14 months of gluten containing diet have a transient gluten intolerance. However we need more experience of this condition before deciding the time for the definite biopsy. The found frequency agrees with that previously reported (10). The initial symptoms are the same as those of coeliac children but the infants with transient gluten intolerance were on average younger at debut. This agrees with the experience of Kuitunen et al (10). The nature of this condition is obscure. It can be a true transient gluten intolerance, it can be a gluten intolerance secondary to a mucosal damage caused by cow's milk (26) or by an infection (2). Finally it can not be excluded that some children have a permanent low grade gluten intolerance giving symptoms in early infancy and taking a longer time for a relapse later in life.

In any case it is important to recognize this condition. This and the existence of symptom free relapses on gluten challenge emphasize the importance of following up these children and of evaluating the effect of gluten challenge with small intestinal biopsy.

Table 1 Main symptoms and signs in children with coeliac disease transient gluten intolerance and unverified and miscellaneous diagnosis

	Group I Coeliac disease	Group II Transient gluten tolerance	Group III Unverified and miscellaneous diagnosis
Failure to thrive	24/34	3/6	6/9
Diarrhoea	26/34	6/6	6/9
Obstipation	3/34	0/6	2/9
Vomiting	9/34	1/6	2/9
Abdominal distension	23/34	1/6	5/9
Rickets	1/34	0/6	0/9

5 children with coeliac disease had normal stools

The first biopsy was taken while the child had a gluten containing diet and the second biopsy after 6–12 months on a gluten free diet. After 1 to 1.5 years on a gluten free diet the child was challenged with gluten. Usually a normal diet was given but in some instances 3 g to 15 g of gluten was added per day to the gluten free diet. The time for the third biopsy depended on the clinical symptoms and if no symptoms appeared the challenge biopsy was taken after 4–6 months on this regime. If no morphological deterioration occurred further biopsies were taken up to two years or longer on a normal diet. Children with a definite clinical relapse within days after gluten challenge began had again a gluten free diet for a month or more. Gluten challenge was then repeated twice. The challenge was regarded as positive if symptoms reappeared each time and it was not considered necessary to perform an intestinal biopsy.

RESULTS

49 children had a severely damaged mucosa in all specimens taken at the first biopsy. They were classified into three groups according to the results of dietary treatment and of the gluten challenge.

Group I Coeliac disease 34 children fulfilled the diagnostic criteria for coeliac disease given above. 11 children had clear clinical symptoms on each gluten challenge and had no challenge biopsy. Of the remaining 23, 13 had no clinical symptoms but all had a moderately to severely damaged mucosa at the challenge biopsy. Table 2 gives the number of births, number of coeliac children each year and the incidence of coeliac disease for the 10 year period. The overall incidence was 1/982 or 1/102 promille. Mean age at debut of symptoms

was 6.9 months and at first biopsy 13 months. 70% of the children were less than one year old at the first biopsy.

Three children were siblings. One boy had a younger sister (born 1976) with coeliac disease. Another boy had a coexisting cystic fibrosis. One girl was the child of immigrant parents.

Group II Transient gluten intolerance Six of the 49 children (12%) did not relapse on gluten challenge and had a normal mucosa or slightly damaged mucosa on a normal diet for two years or more (2, 2, 2.5, 2.5, 4 and 4 years). They had symptoms at 3–8 months old. The mean age at first biopsy was 7.5 months. Initially all became symptomfree on a gluten free but cow's milk containing diet. The second biopsy on this dietary treatment showed definite improvement of mucosal morphology.

Group III Unverified and miscellaneous diagnosis Two had a probable coeliac disease but do not yet fulfill the criteria. Three had a normal mucosa in the challenge biopsy after 5, 9 and 14 months of normal diet. On clinical grounds two had a probable cow's milk protein intolerance. A gluten challenge biopsy was not performed. One boy had a multiple protein intolerance (gluten, cow's milk, egg and fish). One child was lost from the follow up.

DISCUSSION

Family studies of coeliac disease have revealed that asymptomatic cases occur (12, 14

MULTIPLE PITUITARY HORMONE DEFICIENCIES IN EIGHT SIBLINGS OF ONE JEWISH MOROCCAN FAMILY

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ABSTRACT Adler Bier M, Pertzalan A, Laron Z, Lieberman E and Moses S (Institute of Pediatric and Adolescent Endocrinology, Beilinson Medical Center, Petah Tikva, Sackler School of Medicine, Tel Aviv University and Division of Pediatrics, Soroka Medical Center, Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel). Multiple pituitary hormone deficiencies in eight siblings of one Jewish Moroccan family. *Acta Paediatr Scand* 68: 401, 1979. In a Jewish Moroccan inbred family, 8 of 12 siblings were found to have multiple deficiencies of pituitary hormones, including GH, TSH and gonadotrophins. The parents showed no deficiency and are in good health, as are the other 4 siblings. The investigations carried out indicate that in this family the etiology is hereditary in nature, probably being autosomal recessive, with the defect located in the pituitary gland.

KEY WORDS Pituitary hormones, panhypopituitarism, genetic markers

Deficiency of several pituitary hormones including growth hormone is usually found in sporadic cases, often being due to organic disease (9). The familial occurrence of panhypopituitarism, which has been described as having an autosomal recessive mode of inheritance (13), is rare.

Hereby presented is a family in which 8 of 12 siblings were found to be affected by hypopituitarism of varying degrees of expression.

that there were no known familial diseases nor any cases of excessively short stature. There are 12 offspring, the 10th and 11th being twins. All pregnancies and deliveries were uneventful and birth weights were normal although close to the lower limit.

METHOD OF INVESTIGATION

Every family member, with the exception of the 17th child, who was born after completion of this investigation, underwent a thorough clinical examination which in-

FAMILY HISTORY

This family came to our attention upon the referral of female siblings nos. 4 and 5 because of marked growth retardation. They were found to suffer from multiple pituitary hormone deficiencies and questioning of the parents revealed that others of the siblings were also small in stature and furthermore that consanguineal marriages had been frequent throughout the family's history. It was therefore decided to investigate the whole family.

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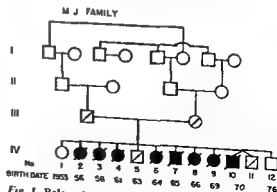


Fig. 1 Relevant parts of the M J family tree as reported by the parents. ■ pituitary insufficiency □ endocrine and genetic markers studies

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REFERENCES

- 1 Alexander J O Dermatitis herpetiformis. In A Rook (ed.) *Major problems in dermatology* vol 4 Saunders Co Ltd London Philadelphia and Toronto 1975 p 236
- 2 Barnes G I & Townley R H W Duodenal mucosal damage in 31 infants with gastroenteritis *Arch Dis Child* 48 343 1973
- 3 Berg N O Borulf S Jakobsson I & Lindberg T How to approach the child suspected of malabsorption *Acta Paediatr Scand* 67 403 1978
- 4 Borgfors N & Selinder P The incidence of coeliac disease in Sweden *Acta Paediatr Scand* 57 260 1968
- 5 Carter C Sheldon W & Walker C The inheritance of coeliac disease *Am Human Genet* 23 266 1949
- 6 Davidson I S P & Fountain J R Incidence of the sprue syndrome with some observations on the natural history *Br Med J* 1 1147 1950
- 7 Fierhird G Frinzén G & Löw B Schizophrenia susceptibility and HLA antigen *Neuropsychology* 1 211 1975
- 8 Ek B Studies on idiopathic non tropical sprue *Acta Med Scand Suppl* 508 1970
- 9 European Society for Pediatric Gastroenterology Diagnostic criteria in coeliac disease *Acta Paediatr Scand* 59 461 1970
- 10 Huittunen P Pulkonen P Perkkio M Savilhti E & Viikari J K Transient gluten intolerance *Acta Paediatr Belg* 30 250 1977
- 11 Lindberg T & Meuwisse G Transient coeliac disease—does it exist? *Acta Paediatr Scand Suppl* 236 p 56 1973
- 12 McCarthy C F Mylotte M Stevens F Egan Mitchell B Fittrell P F & McNicholl B Family studies on coeliac disease in Ireland. In W Th J M Heekens & A S Pena (eds) *Coeliac Disease Proc 2nd Int Coeliac Symposium* H E Stenfort Kroeze Leiden 1974 p 311
- 13 McCrme W M Inheritance of coeliac disease *J Med Genet* 6 129 1969
- 14 MacDonald W C Dobbins W O & Rubin C I Studies of the familial nature of coeliac sprue as biopsy of the small intestine *N Engl J Med* 277 4 1965
- 15 McNerish A S & Anderson C M Coeliac disease in childhood *Clin Gastroenterol* 3 127 1974
- 16 McNerish A S Rolles C J & Arthur L J I Criteria for diagnosis of temporary gluten intolerance *Arch Dis Child* 51 275 1976
- 17 Meuwisse G W Lindberg T & Blomberg M hydraulic multiple intestinal biopsy apparatus for infants *Acta Paediatr Scand* 61 839 1974
- 18 Murrigh T J Reen D J & Gerially J HLA and B9 in coeliac disease *1st Int Symposium HLA and disease* Paris Abstract p 163 1976
- 19 Mylotte M Egan Mitchell B McCarthy C McNicholl B Incidence of coeliac disease in West of Ireland *Br Med J* 1 703 1973
- 20 Nusslé D Bozic C Cox J Deléze G Roux M & Mégevand A Non coeliac gluten intolerance in infancy *3rd Int Coeliac Symposium* Galway 1977
- 21 Picker S M Charlton V Keeling J W Rink R A Ogilvie D Rowlett R J Larcher V F Harris J T Gluten challenge in treated coeliac disease *Arch Dis Child* 53 449 1978
- 22 Shmerling D H Leisinger P & Prader A On the familial occurrence of coeliac disease *Acta Paediatr Scand* 61 501 1972
- 23 Stokes P L Asquith P & Cooke W T Genetic of coeliac disease *Clin Gastroenterol* 2 547 1973
- 24 Strober W Abnormalities of the HLA system in gastrointestinal disease. In J Dusset & A Svejcar (eds) *HLA and disease* Munksgaard Copenhagen 1977 p 168
- 25 Walker Smith J Transient gluten intolerance *Arch Dis Child* 45 523 1970
- 26 Viikari J K & Immonen P Intolerance to cow milk and wheat gluten in the primary malabsorption syndrome in infancy *Acta Paediatr Scand* 56 1967

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MULTIPLE PITUITARY HORMONE DEFICIENCIES IN EIGHT SIBLINGS OF ONE JEWISH MOROCCAN FAMILY

M ADLER BIER A PERTZELAN Z LARON ■ LIEBERMAN* and ■ MOSES†

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ABSTRACT Adler Bier M Pertzelan A Laron Z Lieberman ■ and Moses S (Institute of Pediatric and Adolescent Endocrinology Beilinson Medical Center Petah Tikva Sackler School of Medicine Tel Aviv University and Division of Pediatrics Soroka Medical Center Faculty of Health Sciences Ben Gurion University of the Negev Beer Sheva Israel) Multiple pituitary hormone deficiencies in eight siblings of one Jewish Moroccan family *Acta Paediatr Scand* 68 401 1979 In a Jewish Moroccan inbred family 8 of 12 siblings were found to have multiple deficiencies of pituitary hormones including GH TSH and gonadotrophins The parents showed no deficiency and are in good health as are the other 4 siblings The investigations carried out indicate that in this family the etiology is hereditary in nature probably being autosomal recessive with the defect located in the pituitary gland

KEY WORDS Pituitary hormones panhypopituitarism genetic markers

Deficiency of several pituitary hormones including growth hormone is usually found in sporadic cases often being due to organic disease (9) The familial occurrence of panhypopituitarism which has been described as having an autosomal recessive mode of inheritance (13) is rare

Hereby presented is a family in which 8 of 12 siblings were found to be affected by hypopituitarism of varying degrees of expression

FAMILY HISTORY

This family came to our attention upon the referral of female siblings nos 4 and 5 because of marked growth retardation They were found to suffer from multiple pituitary hormone deficiencies and questioning of the parents revealed that others of the siblings were also small in stature and furthermore that consanguinal marriages had been frequent throughout the family's history It was therefore decided to investigate the whole family

The grandparents of Moroccan origin are first cousins (Fig 1) Both parents were in good health and reported

that there were no known familial diseases nor any cases of excessively short stature There are 17 offspring the 10th and 11th being twins All pregnancies and deliveries were uneventful and birth weights were normal although close to the lower limit

METHOD OF INVESTIGATION

Every family member with the exception of the 17th child who was born after completion of this investigation underwent a thorough clinical examination which in

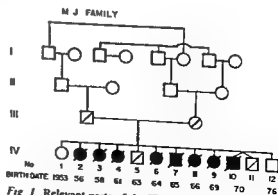


Fig 1 Relevant parts of the M J family tree as reported by the parents ■ pituitary insufficiency □ underwent endocrine and genetic markers studies

Established Investigator of the Chief Scientist's Bureau Ministry of Health

Table 1 Pertinent clinical and endocrinological data of family M J

CA=Chronological age BA=Bone age FBS=Fasting blood sugar TRH=1 μ g one bolus injection 100 μ g/m LH RH 1 μ g one bolus injection 50 μ g/m² ITT=1 μ g one bolus insulin injection 0.1 u/kg

Patient at examination						Serum		
No	Sex	CA (yrs mos)	BA (yrs mos)	Height (cm)	S D	FBS (mg/dl)	hGH (ng/ml)	T ₄ (μ g/dl)
1	Female	18 0	Mature	153.8	-1.3			4.8
2	Female	15 1	10 0	137.5	-3.8	65	2.8	7.8
3	Female	9 4	2 9	100.5	-5.2	84	<2	3.0
4	Female	6 4	2 6	92.2	-5.1	78	<2	2.8
5	Male	12 9	13 0	152.0	Median	70	20.0	4.6
6	Female	10 10	7 0	119.3	-3.5	66	<2	1.7
7	Male	9 8	6 0	114.5	-3.3	64	<2	4.4
8	Female	8 7	7 0	109.6	-3.7	70	2.0	7.8
9	Female	6 0	3 0	94.5	-3.1	70	<2	3.0
10	Male	5 4	3 0	96.5	-2.9	66	3.7	4.0
11	Male	5 4	4 6	112.0	Median	70	20.0	6.1
	Father	49 0		169.0	-0.8	102	>70	5.1
	Mother	38 0		153.0	-1.4	94	14.3	6.8

* hGH peak either after stimulation by insulin or arginine or at sleep (3). * Response after repeated i.m. injections of LH RH 100 μ g/day for 5 days. * Treated with T₄. * Serum levels went further down during hGH therapy.

cluded measurements of body weight and height, X-rays of skull and wrist, electroencephalography, examination of eye fundi and routine testing of urine and blood.

In order to detect a possible linkage between hypopituitarism and genetic markers, blood samples were obtained from the parents and ten of their children (nos 2-11). Test for 18 different genetic loci (6 blood groups, 10 red cell enzymes and 2 serum groups) were carried out.

Hypothalamic-pituitary hormone function was investigated using the following tests: specific gravity of the urine and osmolality of the serum and urine (in all); insulin tolerance test (in parents and patients nos 2-11); arginine infusion test (in sibs nos 2, 3, 4, 10, 11); and sleep growth hormone secretion (4) (in no. 11); serum PBI or T₄ and TRH test (6) (in parents and patients nos 2, 3, 4, 6, 7, 8, 9, 10); and an LH RH test (3) (in parents and patients nos 2-11). It is of note that the TRH test was performed in most subjects prior to the institution of thyroxine treatment and in patients 3 and 4 after discontinuation of treatment which had been given for more than 10 years for a period of 3 weeks. Furthermore, the evaluation of hGH secretion was performed only after a euthyroid state had been achieved.

RESULTS

The pertinent clinical and laboratory data of the children and parents are presented in Table 1. The parents and children nos 1, 5, 11 and 12 (the latter according to the parents)

were of normal height and show normal development. Child no. 6 is mentally retarded. The oldest daughter (no. 1) is married and has two normal children. Eight children (nos 2, 3, 4, 6, 7, 8, 9, 10) were found to have a retardation of growth and skeletal maturation. Girls nos 2, 3 and 4, who have reached pubertal age, show no signs of spontaneous puberty.

In all of the subjects except no. 12, who was not investigated, X-rays showed the skull including the sella turcica to be normal. EEG and eye fundi examinations as well as routine urine and blood tests were all within normal limits. The genetic marker studies were un-revealing. In 12 systems all individuals were found to be of the same type and hence these were regarded as non-informative. In the other 6 potentially informative systems, none of the marker alleles was exclusively characteristic to affected or to non-affected family members, thus a close linkage between the hypopituitarism with any of these systems was ruled out.

The results of the endocrine evaluation can be seen in Table 1.

plasma peak response

RH		LH RH		ITT 110HCS (μ g/dl)	Function of anterior pituitary axes			
SH (U/ml)	Prolactin (ng/ml)	At age (yrs mos)	LH (mU/ml)	FSH (mU/ml)	hGH	Thyroid	Gonadal	Adrenal
08	80	18 9	1.5	2.2	43.0	Deficient	Normal	Normal
39	5.5	18 9	0.7 ^b	1.3 ^b	38.5	Deficient	Deficient	Deficient
5	5.0	15 10	0.5 ^b	0.9 ^b	20.5	Deficient	Deficient	Deficient
		17 9	9.6	2.6	19.7	Normal	Normal	Normal
77	15.0	17 9	0.3	0.5	15.2	Deficient	Deficient	Deficient
68	10.0	11 7	0.7	0.8	23.5	Deficient	Deficient	Deficient
41	10.0	10 6	0.8	0.6	24.5	Deficient	Deficient	Deficient
00	11.0	7 10	1.0	0.6	28.0	Deficient	Deficient	Deficient
07		5 4	0.5	0.5	40.0	Deficient	Deficient	Deficient
		5 4	5.4	1.7	17.5	Normal	Normal	Normal
	77.0		16.7	9.6		Normal	Normal	Normal
	37.0		0.3	2.2		Normal	Normal	Normal

Posterior pituitary

All the subjects tested had normal urine and plasma osmolalities

Anterior pituitary

Adrenal axis All the children tested showed a normal response of the plasma 110HCS to insulin hypoglycaemia

Growth hormone secretion thyroid axis and gonadal axis The parents and children nos 5 and 11 showed normal functions whereas offsprings nos 2 3 4 6 7 8 9 and 10 showed a deficiency of all these hormones. In children nos 3 and 4 the plasma LH and FSH did not rise after repeated i.m. injections of LH RH (100 μ g/day for 5 days)

Prolactin The parents had a normal basal level of plasma prolactin and a normal response to TRH. Among the children studied the basal level and response to TRH were within low normal limits in nos 2 6 7 8 and 9. In siblings 3 and 4 the response of prolactin to TRH was below normal.

DISCUSSION

Several cases of familial occurrence of pan hypopituitarism have been reported in the literature. The hormone deficiency most frequently associated with that of growth hormone is gonadotrophin deficiency followed in order by TSH deficiency and then by partial ACTH deficiency. There is however a variability in the association of these hormone deficiencies in any one family and between different families (12). Hooft & Casneuf (8) described two brothers with hypopituitary dwarfism but in both these cases there had been a prolonged breech delivery. Ferner & Stone (5) reported pituitary dwarfism associated with a very small sella turcica located in a sphenoid bone of abnormal morphology in two sisters. Parks et al (10) described two sisters and one brother with hypopituitarism who had a large sella turcica. Sadeghi Nejad & Senior (14) reviewed the syndrome with genetic aplasia of the anterior pituitary. In all these cases an autosomal recessive mode of inheritance was suggested. Two other reports have suggested

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The results of the endocrine evaluation can be seen in Table 1

EFFECT OF LONG TERM GH ADMINISTRATION ON PITUITARY THYROID FUNCTION IN IDIOPATHIC HYPOPITUITARISM

E. CACCIARI, A. CICOGNANI, P. PIRAZZOLI, F. BERNARDI, F. ZAPPULLA,
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ABSTRACT Cacciari E, Cicognani A, Pirazzoli P, Bernardi F, Zappulla F, Salardi E, Mazzanti L, Biasini A and Valenti E (Department of Paediatrics, University of Bologna, Bologna, Italy). Effect of long term GH administration on pituitary thyroid function in idiopathic hypopituitarism. *Acta Paediatr Scand* 68: 405-409, 1979. — Twenty-four euthyroid children with idiopathic pituitary dwarfism were studied. The euthyroid state for seven of these patients was determined by negative physical examinations, normal plasma T_4 assays and normal ^{125}I uptakes. For the other children, thyroid function was evaluated with T_4 and T_3 assays and on the basis of the TRH test. Each of the children was treated with HGH in one of three different ways. The first group (five cases) was given a HGH dose ranging from 12.4 to 17.2 IU/m²/week. The second and third groups (nine and ten cases, respectively) were treated with 10 and 20 IU/m²/week, respectively. Treatment was carried out for periods ranging from 6 months to 6 years. After no less than 6 months of treatment and at intervals of 6 months (or some multiple of 6 months), plasma T_4 and T_3 assays as well as a TRH test were performed in each patient. In some patients one of the indices was once beyond the upper or lower limit of the normal range (none of the children presented simultaneous abnormal levels of more than one index during the controls). This value, however, returned to within normal limits in the following control. There was no correlation between T_4 and T_3 with the duration of HGH therapy. There was no significant difference between the groups of children treated with the different HGH doses. These data seem to demonstrate that the risk of inducing an alteration in thyroid function in hypopituitary patients during HGH treatment is very slight and that the irregularly abnormal thyroid indices observed in some of the children during one of the controls might be an expression of their metabolic status at that moment.

KEY WORDS Hypopituitarism, HGH therapy, thyroid function.

In idiopathic hypopituitarism it has been demonstrated that the administration of human growth hormone (HGH) can induce alterations in thyroid function (7, 11, 13, 14, 15). In fact, during HGH therapy, a decrease in thyroid uptake (13), a reduction in plasma thyroid hormones (7) and a change in pituitary TSH response (7, 11, 14, 15) have been observed. This last was found to be lower in some cases (7, 11, 14) and higher in others (15). These contradictory results, obtained from a limited number of patients and under experimental conditions that often are not comparable, do not clearly answer the question whether there is a real risk of inducing hypothyroidism during HGH treatment or not.

The purpose of the present study is to clarify this problem.

MATERIALS AND METHODS

Twenty-four prepubertal euthyroid children (15 males and 9 females) with idiopathic pituitary dwarfism were studied. Their chronological age at the time of diagnosis ranged from 3 to 14 years. The euthyroid state for 7 of these patients was confirmed by a negative physical examination, normal plasma T_4 assay and ^{125}I uptake; for the others it was confirmed by T_4 and T_3 determinations and by TSH values before and after TRH (thyrotrophin releasing hormone) administration (1).

Growth hormone function was evaluated by means of insulin-induced hypoglycemia (0.1 U/kg i.v.) and arginine infusion (0.5 g/kg/30 min) (?). Growth hormone was measured utilizing the method of Molinatti et al. (8). For receiving GH therapy, a patient had to have a peak serum

a possible X linked recessive form of hypopituitarism (11, 15)

In the family presented here we were able to demonstrate deficiency of growth hormone secretion and of the hormones of the pituitary thyroid and pituitary gonadal axes. Our finding in 2 children that LH and FSH did not rise after repeated stimulation of the LH RH together with the relatively low response of TSH and prolactin to TRH may point to a primary defect in the pituitary as suggested by Parks et al (10)

Since this disorder is a familial disease it is interesting to speculate as to whether the etiologic factor is a defect in a common hormone precursor or releaser which raises the possibility that a single gene may control more than one pituitary hormone or the etiology may be linked to an anatomic lesion being of the same origin as the syndrome of isolated aplasia of the anterior pituitary (14) but of a minor extent. This could explain the variations in phenotypic expression observed in the various patients. The high incidence of consanguineous marriage in this family and the number of siblings of both sexes affected can only point to a hereditary process of recessive nature.

A recent review on the prevalence of pituitary dwarfism in Israel (2) revealed that the family reported here is the only family in Israel with MPH (Multiple pituitary hormone deficiency and panhypopituitarism).

ACKNOWLEDGEMENT

We would like to thank Sarah Ashbel from the Dept of Human Genetics, Sackler School of Medicine, Tel Aviv University for her laboratory determinations of the genetic markers to Prof. A. Adam and Dr B. Bonne for helpful suggestions.

REFERENCES

1. Blizzard R M & Alberts M. Hypopituitarism by postnatalism and hypogonadism in the newborn infant. *J Pediatr* 48: 782, 1956.
2. Adam A, Josefsberg Z, Pertzalan A, Zadik Z, Chemke J & Laron Z. The prevalence of four types of pituitary dwarfism in Israel. *J Med Genet*. Submitted.
3. Dickerman E, Prager Lewin R & Laron Z. The effect of repeated injections of synthetic LH RH on

the response of plasma LH and FSH in young hypogonadotropic hypogonadal patients. *Fertil Steril* 27: 167, 1976.

4. Fisman C J & Lazarus L. Growth hormone release during sleep in growth retarded children. *Arch Dis Child* 48: 502, 1973.
5. Ferner P E & Stone E F Jr. Familial pituitary dwarfism associated with an abnormal sella turcica. *Pediatrics* 43: 898, 1969.
6. Foley T P Jr, Jacobs L S, Hoffman W, Dauchay W H & Blizzard R M. Human prolactin and thyrotropin concentration in the serum of normal and hypopituitary children before and after the administration of synthetic thyrotropin releasing hormone. *J Clin Invest* 51: 2143, 1977.
7. Greulich W W & Pyle S I. *Radiographic atlas of skeletal development of the hand and wrist*. Stanford University Press, Stanford, California, 1949. 2nd ed.
8. Hooft C & Crisneuf J. Familial hypopituitary dwarfism. *Helv Paediatr Acta* 21: 653, 1966.
9. Laron Z. The hypothalamus and pituitary gland (hypophysis). In H. Hubble (ed.) *Paediatric endocrinology*. Blackwell Sci Publ, Oxford, 1969. pp 35-111.
10. Parks J S, Tenore A, Bongiovanni A M & Kirkland R T. Familial hypopituitarism with large sella turcica. *New Engl J Med* 298: 698, 1978.
11. Phalan P D, Connelley J, Martin F I R, Wertenhall H N B. X linked recessive hypopituitarism. In D. Bergsma (ed.) *Clinical delineation of birth defects*. The endocrine system, birth defects. *Orig Art Ser*, vol VII no 6. The Williams and Wilkins Co. Fourth National Foundation—March of Dimes, Baltimore, 1971. p 24.
12. Rimoin D L, Merimee T J, Rabinowitz D & McKusick V A. Genetic aspects of clinical endocrinology. *Recent Progress Hormone Res* 24: 765, 1968.
13. Rimoin D L & Schimke R N. Genetic disorders of isolated aplasia of the anterior pituitary. Diagnostic studies and treatment in the neonatal period.
14. Sadeghi Nejad A & Senior B. A familial syndrome of isolated aplasia of the anterior pituitary. Diagnostic studies and treatment in the neonatal period. *J Pediatr* 84: 79, 1974.
15. Schimke R N, Spaulding J J & Hollowell J F. X linked congenital panhypopituitarism. In D. Bergsma (ed.) *Clinical delineation of birth defects*. The endocrine system, birth defects. *Orig Art Ser*, vol VII no 6. The Williams and Wilkins Co. Fourth National Foundation—March of Dimes, Baltimore, 1971. p 21.
16. Tanner J M, Whitehouse R H & Takashi M. Standards from birth to maturity for height, weight, height velocity and weight velocity. British children. *Arch Dis Child* 41: 454 and 613, 1966.

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Table 2 T_3 (ng/ml) and T_4 (μ g/100 ml) mean values \pm S D before and after HGH therapy

	Before therapy		After 6 months		After 12 months		After 18 months	
	T_3	T	T_3	T_4	T_3	T_4	T_3	T_4
\bar{x}	1.67	8.05	1.58	6.66 ^a	1.25	7.65	1.73	7.60
S D	0.66	2.14	0.45	1.78	0.33	1.45	0.61	2.16
Range	0.7-7.6	3.9-11.7	1.1-2.7	4.8-9	0.8-1.8	5.5-8.8	0.8-2.7	4.8-10.5
No	17	24	9	10	6	6	7	7
	After 2 years		After 2½ years		After 3 years		After 3½ years	
	T_3	T	T_3	T	T_3	T	T_3	T
\bar{x}	1.78	8.46	1.60	8.93	1.78	8.48	1.90	8.61
S D	0.8	2.27	0.42	1.34	0.46	2.49	0.61	2.01
Range	1.4-7	3.5-14	1.3-4	7.5-17.1	0.9-2.6	5.3-14	1.2-7.5	6.1-12.4
No	12	13	10	10	14	14	7	7
	After 4 years		After 5 years		After 6 years		Controls	
	T_3	T	T_3	T	T_3	T_4	T_3	T
\bar{x}	1.80	8.15	1.0	8.93	1.8	6.7	1.78	8.93
S D	0.78	1.48	0.69	2.45	-	-	0.45	2.24
Range	1.4-7	6.6-9.7	1.3-2.5	6.5-11.4	-	-	0.8-2.7	4.7-14
No	4	4	3	3	1	1	140	217

$p < 0.05$ paired *t* test for the difference with the values before therapy

$p < 0.01$ Student's *t* test for the difference with the controls

$p < 0.05$ Student's *t* test for the difference with the controls

for T_3 in 3 cases for T_4 in 5 cases for basal TSH in 3 cases for TSH area in 5 cases for TSH peak and in 7 cases for TSH maximum increase. During the following control however the altered value always returned to normal.

3) The paired *t* test carried out for all the parameters at the various controls showed a T_3 increase at the 6th month ($p < 0.05$) a decrease in TSH area after TRH at the 12th month ($p < 0.01$) a decrease in basal TSH ($p < 0.05$) and in TSH increase after TRH ($p < 0.01$) at the 24th month.

4) The mean value of the indices of pituitary thyroid function before therapy did not differ from that obtained after HGH treatment (Tables 2 and 3).

5) Whereas mean T_4 at the 6th month ($p < 0.01$) and mean T_3 at the 12th month ($p < 0.05$) were lower maximum TSH increase after TRH appeared to be at the 24th month slightly higher than the control mean value.

6) The percentage of patients having a delayed maximum TSH peak (i.e. occurring at

the 60th minute) did not change significantly during the treatment and was never significantly different from the percentage found in normal children (7.5%).

7) There was no correlation between the T_3 , T_4 and TSH levels with the length of HGH therapy.

8) There were no significant differences among the three groups of patients treated with different HGH dosages. The differences for the various indices were evaluated comparing the mean values obtained before the treatment and at the various controls.

DISCUSSION

A series of alterations in thyroid function linked to the presence of abnormal levels of growth hormone has been reported. In acromegaly both hyperfunction and hypofunction (4, 9) and more recently a lower pituitary response to the TRH stimulus (5, 6) have been described. It has been observed that administration of HGH in patients with GH deficiency

Table 1 Clinical data of 24 patients with idiopathic hypopituitarism

Case	Sex	Chronological age	Associated deficiencies	HGH treatment (IU/m ² /week)	Years of therapy
1 G M	♂	9.5	ACTH	17.2	5
2 P L	♂	13.5	ACTH LH FSH	12.4	4
3 V P	♀	10.6	-	13.5	3½
4 V C	♂	11.3	LH FSH	16.8	6
5 B A	♂	14.0	LH FSH	16	4
6 L A	♂	10.4	-	10	2
7 L D	♂	5.5	-	10	1
8 C D	♂	3.2	-	10	2
9 C P P	♂	10.3	-	10	2
10 A V	♀	11.0	-	10	1½
11 B A	♀	11.0	-	10	1
12 F M	♂	11.9	-	10	1
13 P P	♂	10.6	-	10	1
14 D M V	♂	13.2	-	10	1
15 G M	♀	13.3	-	20	3½
16 M A	♀	9.0	-	20	3
17 G S	♀	6.5	-	20	3½
18 B C	♀	7.4	-	20	3½
19 M S	♂	10.1	-	20	3
20 B F	♂	13.5	-	20	3
21 T S	♀	4.6	-	20	2½
22 F G	♂	5.0	-	20	2½
23 C S	♂	13.7	-	20	1
24 T S	♀	5.7	-	20	1½

growth hormone concentration ≤ 4 ng/ml in response to both tests

LH FSH and ACTH pituitary reserves were investigated by means of an LH RH test ($50 \mu\text{g i.v.}$) and an insulin induced hypoglycemia test (0.1 U/kg i.v.) (2). LH and FSH were assayed according to the method of Reuter et al. (12). Plasma ACTH was measured with the method of Vague et al. (17). Two children (nos. 1 and 2) had an associated ACTH deficiency and others (nos. 2, 4 and 5) had an associated LH FSH deficiency. A subject was considered as having an LH FSH deficiency if during the LH RH test the values of both gonadotrophins were never above the sensitivity of the method (0.5 mIU/ml).

In all patients HGH (Growth Hormone) was administered as follows: Group I (nos. 1-5) 4 IU every other day for a total ranging from 12.4 to 17.2 IU/m²/week; Group II (nos. 6-14) 10 IU/m²/week; Group III (nos. 15-24) 20 IU/m²/week. In the last two groups the HGH dose was divided into three injections per week. The treatment was carried out for periods ranging from six months to six years. During the observation period none of the patients received corticosteroid or gonadotrophin therapy. In all the children regardless of the period of treatment there was an increase in the growth rate of at least 2.5 cm/year as compared to the year preceding treatment. After no less than six months of treatment and at intervals of six months (or multiples of six months) all the children underwent plasma T₃ and T₄ determinations and a TRH test performed as previously described (1) with a 100 μg dose of TRH rapidly injected i.v. and samples collection at 0, 20 and 60 min. The TSH pituitary reserve was stud-

ied by evaluating the peak, the maximum increase and the area under the curve after TRH administration (3). Since a delayed TSH response to TRH may be an expression of a thyroid hypofunction of hypothalamic nature (4) the percentage of children with the TSH peak at the last sample (60 min) was evaluated both before and during HGH treatment. TSH was evaluated according to the method of Odell et al. (10) using the MRC standard 68/38. Plasma T₃ was determined at first by a competitive protein binding analysis (Thiopic 4, Amersham) and more recently by radioimmunoassay (T₃ RIA kit Biodata). Plasma T₄ was evaluated by radioimmunoassay (T₄ RIA kit Biodata).

The statistical analysis of the results was carried out using Student's *t* test, the paired *t* test and chi square as well as by calculating the correlation coefficient *r*.

RESULTS

A summary of the clinical and laboratory data is shown in Tables 1, 2 and 3. The results can be summarized as follows:

- 1) None of the 24 subjects examined presented more than one abnormal index at one control.
- 2) Values above or below the normal range during one of the controls were seen in 1 case.

3) The duration of treatment after the first six months does not seem to influence the levels of the classical parameters of thyroid function. In fact T_3 , T_4 and TSH before and after TRH show no correlation with the duration of the treatment.

4) The HGH dosage does not influence the effect of therapy on pituitary thyroid function. In fact no differences were seen among the three groups of patients treated differently.

5) The temporary irregularly abnormal variations in one of the thyroid indices observed in some of the patients during treatment may be an expression of the metabolic situation in the patient at that moment. As a matter of fact the evaluation of pituitary thyroid indices in a normal child on different days may give completely different results (our unpublished data).

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REFERENCES

- Cacciani E, Bernardi F, Salardi S, Tassoni P, Cicognani A, Pirazzoli P, Zappulla F & Zingoni S. The thyrotropin releasing hormone test in idiopathic pituitary dwarfism. *Helv Paediatr Acta* 29: 481, 1974.
- Cacciani E, Cicognani A, Tassoni P, Pirazzoli P, Bernardi F, Salardi S & Zappulla F. Problemi e risultati nella diagnosi e nella terapia delle iposomie con deficit dell'ormone dell'accrescimento. *Minerva Pediatr* 26: 1209, 1974.
- Cacciani E, Cicognani A, Pirazzoli P, Tassoni P, Zappulla F, Salardi S & Bernardi F. Relationship among the secretion of ACTH, GH and cortisol during the insulin induced hypoglycemia test in the normal and obese child. *J Clin Endocrinol Metab* 40: 80, 1975.
- Codaccioni J L, Jaquet P, Brismuth J & Freychet J. La thyroïde dans l'acromégalie. *Ann Endocrinol* 3: 68, 1971.
- Faglia G, Beck Pecoloz P, Ferrati C, Ambrosi M, Spada A, Travaglini P & Paracchi S. Plasma thyrotropin response to TRH in patients with pituitary and hypothalamic disorders. *J Clin Endocrinol Metab* 37: 595, 1973.
- Kastin A J, Shally A V, Gonzales Barcena D, Schalch D S & Lee L. Different responses to two hypothalamic hormones in acromegals. *Arch Intern Med* 130: 973, 1970.
- Lippe B M, Van Herle A J, Lanfranchi S H, Uller R B, Lavin N & Kaplan S A. Reversible hypothyroidism in growth hormone deficient children treated with human growth hormone. *J Clin Endocrinol Metab* 40: 612, 1975.
- Molnatti G M, Massara F, Pennisi F, Scascelati G, A Strumia M & Vancheri L. Radioimmunoassay of growth hormone. *J Nucl Biol Med* 13: 76, 1969.
- Muktar E, Wilkinson R, Alexander L, Appleton D & Hall R. Thyroid function in acromegaly. *Lancet* ii: 79, 1971.
- Odell W D, Wilber J F & Paul W M. Radioimmunoassay of thyrotropin in human serum. *J Clin Endocrinol Metab* 25: 1179, 1965.
- Porter B A, Refetoff S, Rosenfield R L, De Groot L J, Fang V S & Stark V. Abnormal thyroxine metabolism in hypsomatotropic dwarfism and inhibition of responsiveness to TRH during GH therapy. *Pediatrics* 51: 668, 1973.
- Reuter A M, Hendrick J C & Franchimont P. Mise au point d'un dosage radioimmunologique rapide des gonadotrophines. *Ann Biol Clin* 31: 479, 1973.
- Root A W, Bongiovanni A M & Eberlein W R. Inhibition of thyroidal radioiodine uptake by human growth hormone. *J Pediatr* 76: 472, 1970.
- Root A W, Snyder P J, Rezvani I, Di George A M & Unger R D. Inhibition of thyrotropin releasing hormone mediated secretion of thyrotropin by human growth hormone. *J Clin Endocrinol Metab* 36: 103, 1973.
- Sato T, Suzuki Y, Taketani T, Ishiguro K, Masuyama T, Takata I, Sano M, Kawashima H, Koizumi D & Nakajima H. Enhanced peripheral conversion of thyroxine to triiodothyronine during HGH therapy in GH deficient children. *J Clin Endocrinol Metab* 45: 34, 1977.
- Snyder P J & Unger R D. Inhibition of thyrotropin releasing hormone by small quantities of thyroid hormones. *J Clin Invest* 51: 787, 1972.
- Vague P, Oliver C, Jaquet P & Vague J. Le dosage de l'ACTH plasmatique. Résultats chez les sujets normaux. *Rev. Eur. Etud. Clin. Biol.* 16: 485, 1971.
- Vignen R, Squatrito S, Pezzino V, Filetti S & Polosa P. The effect of short term triiodothyronine administration on thyroxine response to exogenous TSH in man. *J Clin Endocrinol Metab* 41: 974, 1975.

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Table 3 Mean values \pm S.D. of basal peak area and maximum TSH (μ U/ml) increase during the TRH test (100 μ g i.v.)

	Basal value	Peak	Area	Increment	Basal value	Peak	Area	Increment
Before HGH therapy (n=17)					After 6 months of therapy (n=10)			
x	5.76	16.70	39.79	10.94	3.24	13.53	31.96	10.79
S.D.	4.51	7.93	21.65	6.39	2.37	4.89	11.79	4.59
Range	0.8-14	6.9-43.5	15.4-114	4.2-30.1	0.8-8.8	4.6-20.5	11.6-52	3.8-17.8
After 12 months of therapy (n=6)					After 18 months of therapy (n=7)			
x	4.45	13.80	30.63*	9.35	4.84	13.47	37.86	8.53
S.D.	1.89	4.48	11.67	4.86	3.49	4.25	10.48	4.98
Range	2.5-8	9.1-20	21.5-51	4.4-15.7	0.8-10.2	8.5-18.8	20.7-47	2.2-13.6
After 24 months of therapy (n=13)					After 24 months of therapy (n=10)			
x	3.44	20.09*	46.96	16.55	3.96	18.64	40.45	14.69
S.D.	1.60	10.71	23.72	10.36	2.91	8.82	19.21	7.84
Range	1.2-7.7	8.2-40	20.6-92	4.6-37.3	0.6-9	3.2-33.6	6.7-75	2.7-25.8
After 3 years of therapy (n=14)					After 3 years of therapy (n=7)			
x	4.62	15.79	37.06	11.13	11.19	16.53	41.54	10.44
S.D.	2.46	7.35	17.68	6.63	4.26	10.42	25.95	11.16
Range	0.8-10.2	7.4-34	19.8-82	2.8-29.6	0.8-14.6	2.5-36.5	5.6-91.6	0.6-13.8
After 4 years of therapy (n=4)					After 5 years of therapy (n=3)			
x	6.45	16.95	36.23	9.75	6.23	12.20	30.80	5.97
S.D.	4.41	6.38	13.86	4.61	5.89	7.93	23.36	2.34
Range	2.6-12	11.2-26	29-57.1	3.2-14	2.2-13	5.6-21	14-37.5	3.4-8
After 6 years of therapy (n=1)					Controls (n=80)			
x	1.6	6.7	16.9	5.1	4.64	16.27	38.00	11.63
S.D.	-	-	-	-	2.53	7.09	15.83	6.40
Range	-	-	-	-	0.6-10.2	5.5-36.8	11.4-86	3.2-30.5

* $p < 0.05$ paired t test for the difference with the values before treatment* $p < 0.01$ paired t test for the difference with the values before treatment

may diminish 131 I uptake by the thyroid (13) may lower plasma level of the total T_4 free T_4 and T_3 (7) or on the contrary may increase plasma T_3 level with a normal T_4 level (14). Porter et al. (11) found that the decreased TSH response to TRH after a few days of HGH therapy became normal again after some months of treatment. These data contrast with the results obtained by Lippe et al. (7) who found (in 3 out of 6 patients) a decrease in the response to TRH after 6 months of treatment. Sato et al. (15) found an increase in the response to TRH after 3-12 months of therapy. These contradictory results may be due to the low number of cases studied, to the different parameters taken into consideration and to the different experimental conditions.

Our investigation carried out on a substan-

tial number of subjects treated with different HGH doses and for periods of time ranging from six months to six years seems to demonstrate the following:

1) The risk of inducing a significant alteration of thyroid function in the growth hormone deficient patient by HGH treatment is evidently very slight.

2) If some alterations in the thyroid function are induced by the HGH administration, these are most likely to occur during the first months of therapy, i.e. during the period we did not investigate, and they must be reversible. This is also demonstrated by the fact that during the first three months of HGH therapy a faster conversion of T_4 into T_3 is reported (15); this can lead to a decrease in T_4 (15), in 131 I uptake (18) and in TSH response to TRH (16).

3) The duration of treatment after the first six months does not seem to influence the levels of the classical parameters of thyroid function. In fact T_3 , T_4 and TSH before and after TRH show no correlation with the duration of the treatment.

4) The HGH dosage does not influence the effect of therapy on pituitary thyroid function. In fact no differences were seen among the three groups of patients treated differently.

5) The temporary irregularly abnormal variations in one of the thyroid indices observed in some of the patients during treatment may be an expression of the metabolic situation in the patient at that moment. As a matter of fact the evaluation of pituitary thyroid indices in a normal child on different days may give completely different results (our unpublished data).

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REFERENCES

- Cacciani E, Bernardi F, Salardi S, Tassoni P, Cicognani A, Pirazzoli F & Zingoni S. The thyrotropin releasing hormone test in idiopathic pituitary dwarfism. *Helv Paediat Acta* 29: 481 (1974).
- Cacciani E, Cicognani A, Tassoni P, Pirazzoli P, Bernardi F, Salardi S & Zappulla F. Problemi e risultati nella diagnosi e nella terapia delle iposomie con deficit dell'ormone dell'accrescimento. *Minerva Pediatr* 26: 109 (1974).
- Cacciani E, Cicognani A, Pirazzoli P, Tassoni P, Zappulla F, Salardi S & Bernardi F. Relationships among the secretion of ACTH, GH and cortisol during the insulin induced hypoglycemia test in the normal and obese child. *J Clin Endocrinol Metab* 40: 80 (1975).
- Codaccioni J L, Jaquet P, Bismuth J & Freychet P. La thyroïde dans l'acromégalie. *Ann Endocrinol* 37: 768 (1971).
- Faglia G, Beck Pecoz P, Ferrari C, Ambrosio B, Spada A, Travagli M & Paracchi S. Plasma thyrotropin response to TRH in patients with pituitary and hypothalamic disorders. *J Clin Endocrinol Metab* 37: 595 (1973).
- Kastin A J, Shally A V, Gonzales Barcena II, Schalch D S & Lee L. Different responses to two hypothalamic hormones in acromegals. *Arch Intern Med* 130: 973 (1971).
- Lippe B M, Van Herle A J, Lanfranchi S H, Uller R P, Lavin N & Kaplan S A. Reversible hypothyroidism in growth hormone deficient children treated with human growth hormone. *J Clin Endocrinol Metab* 40: 617 (1975).
- Molnatti G M, Massara F, Pennisi F, Scasselatti G A, Strumia B & Vancheri L. Radioimmunoassay of growth hormone. *J Nucl Biol Med* 13: 76 (1969).
- Muktar E, Wilkinson R, Alexander L, Appleton III & Hall R. Thyroid function in acromegaly. *Lancet* II: 79 (1971).
- Odell W D, Wilber J F & Paul W E. Radioimmunoassay of thyrotropin in human serum. *J Clin Endocrinol Metab* 25: 1179 (1965).
- Porter H A, Refetoff S, Rosenfield R L, De Groot L J, Fang V S & Stark V. Abnormal thyroxine metabolism in hypsomatotropic dwarfism and inhibition of responsiveness to TRH during GH therapy. *Pediatrics* 51: 668 (1973).
- Reuter A M, Hendrick J C & Franchimont P. Mise au point d'un dosage radioimmunologique rapide des gonadotrophines. *Ann Biol Clin* 31: 479 (1973).
- Root A W, Bongiovanni A M & Eberlein W R. Inhibition of thyroidal radioiodine uptake by human growth hormone. *J Pediatr* 76: 42 (1970).
- Root A W, Snyder P J, Rezvani I, Di George A M & Utiger R D. Inhibition of thyrotropin releasing hormone mediated secretion of thyrotropin by human growth hormone. *J Clin Endocrinol Metab* 36: 103 (1973).
- Sato T, Suzuki Y, Taketani T, Ishiguro K, Masuyama T, Takata I, Sano M, Kawashima H, Koizumi D & Nakajima H. Enhanced peripheral conversion of thyroxine to triiodothyronine during HGH therapy in GH deficient children. *J Clin Endocrinol Metab* 45: 374 (1977).
- Snyder H J & Utiger R D. Inhibition of thyrotropin releasing hormone by small quantities of thyroid hormones. *J Clin Invest* 51: 707 (1973).
- Vague P, Oliver C, Jaquet P & Vague J. Le dosage de l'ACTH plasmatique. Résultats chez les sujets normaux. *Rev Eur Etud Clin Biol* 16: 483 (1971).
- Vigneri R, Squatrito S, Pezzino V, Filetti R & Polosa P. The effect of short term triiodothyronine administration on thyroxine response to exogenous TSH in man. *J Clin Endocrinol Metab* 41: 974 (1975).

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CHANGES IN SERUM CONCENTRATIONS OF THYROID HORMONES AND THYROID HORMONE BINDING PROTEINS DURING EARLY INFANCY

Studies in Healthy Fullterm Small for gestational Age and Preterm Infants Aged 7 to 240 Days

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ABSTRACT Jacobsen B B and Hummer L (University Clinic of Paediatrics Children's Hospital Fuglebakken and the Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark) Changes in serum concentrations of thyroid hormones and thyroid hormone binding proteins during early infancy Studies in healthy fullterm small for gestational age and preterm infants aged 7 to 240 days *Acta Paediatr Scand* 68 411 1979 — Serum concentrations of thyrotropin (TSH) thyroxine (T_4) triiodothyronine (T_3) thyroxine binding globulin (TBG) prealbumin (TBPA) and albumin (Alb) were determined in 492 blood samples from 127 fullterm (FT) 91 small for-gestational age (SGA) and 114 preterm (PT) healthy infants aged 7 to 240 days Serum T_4 decreased about 20% during the first month of life In infants aged 7-49 days serum T_4 concentrations were significantly lower in SGA than in FT infants and even lower values were found in PT infants Serum T_3 increased 50-70% reaching maximal values by 50-79 days of life Serum T_3 levels were higher in FT than in SGA infants throughout the observation period In PT infants serum T_3 increased from low values to levels which exceeded those of SGA and FT infants by 120-240 days of life Serum TSH level did not change with age and was ≤ 5 mU/l in all infants Serum TBG values were high compared to normal adult values and did not change significantly with age Comparable serum TBG values were found in FT SGA and PT infants Serum TBPA increased with age Serum TBPA increased gradually in FT infants In SGA infants serum TBPA increased from low values to levels which by 120-240 days of life exceeded those of PT and FT infants In PT infants a decrease in serum TBPA appeared before the rise commenced Serum Alb increased gradually in FT SGA and PT infants during the observation period Serum Alb in PT infants aged 30-119 days was lower than those in FT infants with similar ages These physiological changes in serum concentrations of thyroid hormones and hormone binding proteins during early infancy should be considered when interpreting thyroid function tests in infants with various maturity

KEY WORDS Thyroxine triiodothyronine thyrotropin thyroxine binding globulin prealbumin albumin infants

During the first week of life pronounced changes in serum concentrations of thyrotropin (TSH) and thyroid hormones occur (9 16) Later in infancy and childhood serum TSH remains constant at the level of normal adults (1 3 11) whereas serum protein bound iodine and thyroxine (T_4) show a progressive decrease with age (5 6 11 22 24) Serum triiodothyronine (T_3) levels are increased in infants aged 1-6 months compared with cord

blood levels (1 8 14 18 21) but the postnatal alterations have not been completely elucidated Very little is known about the thyroid hormone binding protein concentrations in infancy A higher binding capacity of thyroxine binding globulin (TBG) and a lower binding capacity of thyroxine binding prealbumin (TBPA) in newborn infants compared with those in older children and adults have been reported (7 21)

Table 2 Median and range of serum concentrations of thyroxine and triiodothyronine in full term (FT), small for gestational age (SGA) and preterm (PT) infants with age ranging from 7-240 days

(n)=number of blood samples. Range indicates lowest and highest values

	Postnatal age in days					
	7-13	14-29	30-49	50-79	80-119	120-240
Serum T_4 (nmol/l)						
FT						
Median	17	149	143	16	143	131
Range	95-83	85-260	75-196	64-198	95-197	64-170
(n)	(67)	(35)	(33)	(36)	(18)	(19)
SGA						
Median	165	138	126	129	128	108
Range	101-270	88-219	90-180	91-167	93-187	82-178
(n)	(31)	(37)	(25)	(19)	(11)	(17)
PT						
Median	143	122	118	107	127	111
Range	84-216	77-221	59-237	50-193	90-211	82-148
(n)	(74)	(47)	(23)	(19)	(14)	(17)
	$p < 0.01$	$p < 0.01$	$p < 0.01$	N S	N S	N S
Serum T_3 (nmol/l)						
FT						
Median	00	2.47	2.69	3.10	2.97	2.34
Range	1.16-3.49	1.62-5.07	1.77-5.04	1.80-4.72	2.11-7.50	1.16-3.77
(n)	(34)	(18)	(19)	(19)	(10)	(19)
SGA						
Median	2.04	1.11	2.23	2.61	2.55	2.32
Range	1.3-2.91	1.78-3.11	1.44-3.49	1.0-3.33	1.87-3.54	1.47-3.18
(n)	(16)	(6)	(11)	(17)	(17)	(17)
PT						
Median	1.77	1.72	2.04	2.88	2.67	3.00
Range	1.29-2.85	0.66-3.00	0.30-3.35	1.03-3.88	1.88-3.89	2.16-3.67
(n)	(17)	(76)	(12)	(13)	(13)	(17)
	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.05$

p is the level of significance for the Kruskal Wallis test. N S = not significant

Thyrotropin concentrations

Serum TSH concentrations remained essentially constant during infancy and were similar in FT, SGA and PT infants (≤ 5 mU/l).

Thyroid hormone concentrations

Serum T_4 concentrations decreased during the first month of life in all maturity groups (Fig. 1). The T_4 decrease was about 20%. In infants aged 7 to 49 days serum T_4 levels were significantly lower in SGA than in FT infants and even lower values appeared in PT infants (Table 2). Later comparable serum T_4 levels

were found in the three groups of infants.

Serum T_3 concentrations increased to maximal values at 50-79 days of life (Fig. 1). The T_3 increase was 50-70%. The serum levels of T_3 in FT, SGA and PT differed significantly throughout the study period (Table 2). From 7 to 49 days of life the lowest serum T_3 level appeared in PT infants, but later serum T_3 level in PT exceeded those of SGA and FT infants.

The ratios between serum T_4 and serum T_3 concentrations (T_4/T_3) decreased during the study period (from 85 to 45 (median values)).

Table 1 *Body weights (g) in fullterm (FT) small for gestational age (SGA) and preterm (PT) infants*

Median and range values in each age group are presented range indicates lowest and highest values (n)=number of blood samples

	Postnatal age in days					
	7-13	14-29	30-49	50-79	80-119	120-240
FT						
Median	3 335	3 560	4 250	5 050	5 705	7 170
Range	2 590-4 250	2 660-4 400	3 000-6 500	3 550-7 670	4 350-8 900	5 650-9 700
(n)	(62)	(35)	(14)	(36)	(18)	(21)
SGA						
Median	2 420	2 745	3 250	3 780	4 805	6 590
Range	1 760-2 980	2 000-3 680	2 520-5 300	2 910-4 780	4 150-6 000	3 650-7 830
(n)	(33)	(38)	(25)	(70)	(12)	(17)
PT						
Median	2 240	2 415	2 820	3 440	4 470	6 050
Range	1 620-2 850	1 000-3 310	1 210-3 670	1 520-4 710	2 070-6 000	3 670-8 450
(n)	(24)	(42)	(23)	(19)	(21)	(17)
	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.001$	$p<0.01$	$p<0.0^*$

p is the level of significance for the Kruskal Wallis test

Recently we reported data indicating different serum levels of thyroid hormones and hormone binding proteins in fullterm (FT) small for gestational age (SGA) and preterm (PT) babies during the first week of life (16, 17). The present report is intended to characterize serum concentrations of TSH, thyroid hormones and thyroid hormone binding proteins in healthy babies later in infancy.

MATERIALS AND METHODS

A total number of 306 euthyroid infants with ages ranging from 7 to 240 days were studied. The infants were in good clinical condition without infectious respiratory distress or treatment which could influence serum thyroid hormone levels. Gestational age and maturity were assessed as reported previously (15). The fullterm infants group consisted of 127 infants with gestational ages from 37-42 weeks and birth weights 2750-4350 g. The SGA infant group consisted of 91 infants with gestational ages from 37-41 weeks and birth weights 1790-2750 g. Finally 88 infants were preterm infants with gestational ages from 25-36 weeks and birth weights from 830-2750 g.

A total number of 492 blood samples were collected i.e. 1 to 6 samples per infant. Blood was drawn for reasons other than clinically suspected thyroid dysfunction. Informed parental consent was obtained. Blood was drawn from a peripheral vein and serum frozen at -20°C until analyses were performed. Blood sampling was not complete for determination of all thyroid variables only

serum T_4 concentrations were measured in nearly all infants (see Table 1). Usually ≥ 10 determinations of the variables were obtained in each age group.

Serum concentrations of TSH and T_3 were measured by radioimmunoassay and serum T_4 was determined by competitive binding technique (15). Serum concentrations of TBG, TBPA and albumin (Alb) were determined using rocket immunoelectrophoresis as reported previously (17). The protein concentrations were presented in terms of those obtained in a reference serum (arbitrarily set to 100 units per liter). The absolute serum TBG, TBPA and Alb concentrations in the reference serum are 93 mg/l, 297 mg/l and 43 000 mg/l respectively (17).

In the statistical calculations nonparametric tests were used (25). The Kruskal Wallis test was used to decide whether the differences between thyroid variables in FT, SGA and PT infants signify population differences or represent merely chance variations. The Wilcoxon test was used to determine whether variables in two groups of infants were different. The null hypothesis was rejected at the 0.05 level of significance.

RESULTS

Body weights

Table 1 shows median and range values of body weights in each age group. The body weights of FT, SGA and PT infants differed significantly throughout the study period. The median increase in body weight was 120% in FT and 170% in low birth weight infants.

Table 3 Median and range of serum concentrations of thyroxine binding proteins (TBG TBPA Albumin) in fullterm (FT) small for gestational age (SGA) and preterm (PT) infants
(n)=number of blood samples. Range indicates lowest and highest values

	Postnatal age in days					
	7-13	14-29	30-49	50-79	80-119	120-140
<i>Serum TBG (arb u/l)</i>						
FT						
Median	163	190	188	195	191	198
Range	171-187	135-19	91-268	112-96	138-285	133-268
(n)	(15)	(10)	(13)	(19)	(11)	(14)
SGA						
Median	169	167	167	164	170	145
Range	155-17	90-220	130-207	116-219	111-218	108-211
(n)	(11)	(19)	(20)	(15)	(10)	(12)
PT						
Median	170	166	179	155	199	186
Range	151-200	100-210	100-251	76-230	96-267	177-255
(n)	(17)	(20)	(10)	(15)	(11)	(10)
	N S	N S	N S	N S	N S	$p<0.05$
<i>Serum TBPA (arb u/l)</i>						
FT						
Median	37	44	44	51	48	52
Range	14-46	17-51	21-61	4-73	21-70	27-78
(n)	(13)	(7)	(11)	(14)	(8)	(15)
SGA						
Median	27	29	34	43	54	75
Range	19-71	15-53	17-68	20-59	33-70	23-104
(n)	(9)	(11)	(16)	(13)	(9)	(11)
PT						
Median	30	8	21	39	49	59
Range	19-41	18-47	1-32	16-62	34-61	26-88
(n)	(9)	(17)	(7)	(14)	(9)	(10)
	N S	N S	$p<0.01$	$p<0.05$	N S	N S
<i>Serum Alb (arb u/l)</i>						
FT						
Median	8	92	9	92	104	99
Range	66-94	71-97	85-166	76-127	83-145	73-110
(n)	(14)	(8)	(13)	(14)	(11)	(13)
SGA						
Median	77	84	89	92	96	97
Range	69-96	63-104	76-105	79-109	89-120	67-111
(n)	(11)	(19)	(18)	(14)	(10)	(12)
PT						
Median	85	80	84	88	92	97
Range	55-106	63-96	4-105	42-95	48-10	82-103
(n)	(11)	(18)	(10)	(14)	(8)	(9)
	N S	N S	$p<0.01$	N S	N S	N S

N S = not significant

cantly lower than those in FT infants with similar ages ($p<0.05$). Only in age group 30-49 days serum Alb in SGA infants differed significantly from those in FT and PT babies

DISCUSSION

The present data indicate that pronounced changes in serum concentrations of thyroid hormones and hormone binding proteins oc

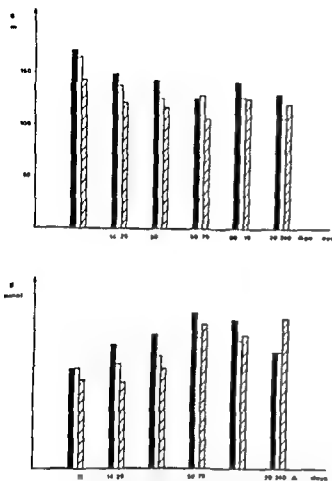


Fig. 1 Median values of serum thyroxine (T₄) and triiodothyronine (T₃) concentrations in fullterm (closed bars), small for gestational age (open bars) and preterm (hatched bars) infants ranging in age from 7 to 240 days.

Thyroid hormone binding protein concentrations (Fig. 2)

Median and range values of serum TBG, TBPA and Alb concentrations are shown in Table 3. The *p* values of the Kruskal-Wallis test are also given. A wide range in serum protein concentrations was seen in all age groups. Serum TBG level did not change significantly with age, but great variations were seen particularly in PT infants. The serum TBG levels in FT, SGA and PT infants did not differ significantly except in infants aged 120–240 days (Table 3). Serum TBPA increased with age (Table 3); the increases were more pronounced in low birth weight infants (100–175%) than in FT infants (40%). In SGA infants serum TBPA increased from low levels to levels exceeding those of FT infants at 120–

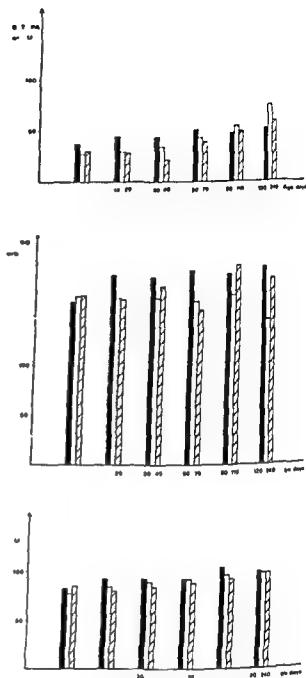


Fig. 2 Median values of serum concentrations of thyroxine binding globulin (TBG), prealbumin (TBPA) and albumin (Alb) in fullterm (closed bars), small for gestational age (open bars) and preterm (hatched bars) infants ranging in age from 7 to 240 days.

240 days of life ($p < 0.05$). In PT infants serum TBPA decreased initially before the TBPA rise commenced.

Serum Alb concentrations in FT as well as in low birth weight infants presented a gradual increase (about 20–25%) (Table 3). Serum Alb in PT infants aged 30–119 days were signifi-

TBG and TBPA concentrations associated with idiopathic respiratory distress syndrome (18).

During the first week of life serum concentrations of TBG, TBPA and Alb differ significantly in FT, SGA and PT infants (17) and the present data indicate that thyroid hormone binding protein levels also later in infancy are influenced by the maturity. A tendency towards higher serum TBG levels in FT than in low birth weight newborns was seen. Serum TBPA in SGA infants increased from very low levels to TBPA levels which exceeded those of FT infants aged 120–240 days (Fig. 2). In PT babies a decrease in serum TBPA level appeared initially after which a pronounced increase occurred. Serum albumin level tended to be higher in FT than in SGA and PT infants but the postnatal serum albumin increases were of the same magnitude in the three groups of infants. Similar findings in mature babies have been reported previously (20). These postnatal changes in serum protein concentrations probably reflect alterations and differences in synthesis and metabolism of TBG, TBPA and Alb in FT, SGA and PT infants. Several factors influence the protein metabolism (23) but the mechanisms that determine these changes in early infancy as well as later in childhood (10–11) are unknown.

Knowledge of these postnatal alterations in serum levels of thyroid hormones and hormone binding proteins seems to be of clinical significance. The changes in serum T_4 and T_3 concentrations have to be considered in diagnosis and treatment of hypothyroidism in early infancy. It is also important to keep the differences between FT, SGA and PT infants in mind when interpreting the *in vitro* thyroid function tests in infants of various maturity.

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REFERENCES

- Aboud J M, Klein A H, Foley T P & Larsen P ■ Total and free triiodothyronine and thyroxine in early infancy. *J Clin Endocrinol Metab* 39: 263, 1974.
- Bernal J & Refetoff S The action of thyroid hormone. *Clin Endocrinol* 6: 777, 1977.
- Binet E, Sizzenko P C & Job J C Résultats du dosage radioimmunologique de la thyroestimuline hypophysaire (TSH) dans le plasma chez l'enfant normal et dans les hypothyroïdies congénitales. *Arch Fr Pédiatr* 28: 179, 1971.
- Brien T G, Fay J A & Griffin E A Thyroid status in the newborn infant: Effective thyroxine ratio and free thyroxine index. *Arch Dis Child* 49: 225, 1974.
- Chadd M A, Davies D F & Gray O P Protein bound iodine in premature infants. *Arch Dis Child* 43: 217, 1968.
- Danowski T S, Johnston S Y, Price W C, McKelvy M, Stevenson S S & McCluskey E R Protein bound iodine in infants from birth to one year of age. *Pediatrics* 7: 240, 1951.
- De Nayer P, Van Den Schneck H ■ & De Visser M Le transport sérique de la thyroxine chez l'enfant. *Ann Endocrinol (Paris)* 26: 360, 1965.
- Dutau G, Bayard F, Bennet P & Rochuccioli P Dosage radioimmunologique de la triiodothyronine plasmatique chez l'enfant normal et dans différentes circonstances pathologiques. *Arch Fr Pédiatr* 31: 861, 1974.
- Erenberg A, Phelps D L, Lam R & Fisher D A Total and free thyroid hormone concentrations in the neonatal period. *Pediatrics* 53: 711, 1974.
- Fisher D A & Dussault J H Development of the mammalian thyroid gland. In M A Greer & D H Solomon (eds.) *Handbook of physiology: Endocrinology III: Thyroid*. American Physiological Society, Washington, 1974, p. 21.
- Fisher D A, Sack J, Oddie T H, Pekary A E, Hershman J M, Lam R W & Parslow M E Serum T_4 , TBG, T_4 uptake, T_4 reverse T_4 and TSH concentrations in children 1 to 15 years of age. *J Clin Endocrinol Metab* 45: 191, 1977.
- Hays G C & Muirhead J E Normal free thyroxine index values in infancy and childhood. *J Clin Endocrinol Metab* 39: 958, 1974.
- Hesch R D, Gatz J, Juppner H & Stubbe P TBG-dependency of age related variations of thyroxine and triiodothyronine. *Horm Metab Res* 9: 141, 1977.
- Homoki J, Loos U, Rothenbuchner G, Grimm V & Teller W M TSH, T_4 , T_3 and reverse T_3 .

cur in early infancy. These changes are related to the postnatal age as well as to the maturity of the infants.

Earlier observations of a decrease in total serum T_4 and protein bound iodine concentrations during infancy (1, 4, 5, 6, 12, 19, 21, 22, 24) were confirmed by the present study. A gradual decrease in serum T_4 occurred during the first month of life in all groups of infants. During the first week serum T_4 concentrations were lower in SGA than in FT infants and even lower values appeared in PT infants (16). The present study indicates that these significant differences continue until 30–49 days of age.

Previous studies have demonstrated higher serum T_3 concentrations in infants aged 1–6 months than in newborns (1, 8, 14, 21) but detailed information of the postnatal changes were not given. In the present study a gradual increase in total serum T_3 levels was found in FT and SGA infants from 7–13 days of age to 50–79 days of age, after which a decrease in median values appeared in FT and SGA infants. The maximal serum T_3 values were higher than serum T_3 levels of normal adults (normal range 1.10–2.40 nmol/l). The T_3 increase seemed to be more pronounced in FT than in SGA infants. In PT infants the T_3 increase was delayed because serum T_3 values in PT infants aged 7–13 days and 14–29 days were similar in accordance with a recent report by Homoki et al. (14). Later serum T_3 in PT infants increased and exceeded T_3 levels of SGA and FT infants by 120–240 days of life. In that way serum T_3 concentrations in FT, SGA and PT infants differed significantly throughout the study period.

The results of present and previous studies (1, 9, 16) indicate a *diphase* pattern in total serum T_3 concentrations during early infancy. After the presumably TSH induced increase in free and total serum T_3 to peak values by 24–48 hours of life a decrease occurs. Later serum T_3 again increases to maximal values at 50–79 days of age but in all groups of infants serum TSH concentrations remained ≤ 5 mU/l.

Enhanced peripheral T_4 to T_3 conversion is suggested as one cause of the increase in serum T_3 contemporary with the serum T_4 decrease a conversion which may be different in FT, SGA and PT infants. However variations in secretion of T_3 and T_4 from the thyroid gland influenced by age and maturity may also be of significance. Since most of the extra-thyroidal pool of T_4 and T_3 is located in the intracellular compartment (2) the present data probably reflect pronounced postnatal changes in thyroid hormone metabolism. In this connection it is noteworthy that the increase in body weight was comparable in SGA and PT infants and more pronounced than in FT babies.

Serum TBG levels did not change significantly whereas serum TBPA and Alb increased in all groups of infants. The decrease in serum T_4 was quantitatively more pronounced than the T_3 increase and the data indicate a decrease in the saturation of hormone binding sites upon the serum proteins during early infancy. This could also explain some of the age related changes in T_3 resin uptake and other *in vitro* tests reported previously (4, 17, 19, 22).

Very little information of serum levels of thyroid hormone binding proteins during infancy is available. Measurement of serum T_4 binding capacity has shown high TBG and low TBPA binding capacities in infants in comparison with those in adults (7). Using radioimmunoassay Hesch et al. (13) found increased serum TBG concentrations in infants and older children but the number of samples was rather small. Montalvo et al. (21) observed a decrease in serum TBG binding capacity between one week and one month of age in mature infants which was not confirmed by the present data. However the median values of serum TBG concentrations tended to be lower in FT and SGA and higher in PT infants in comparison with the TBG values of healthy infants during the first week of life reported previously (17). Recently we reported pronounced laterations in serum

PHARMACOKINETICS OF AMIKACIN IN INFANTS AND PRE SCHOOL CHILDREN

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ABSTRACT Kafetzis D A Sinaniotis C A Papadatos C J and Kosmidis J (Second Department of Paediatrics and First Department of Propedeutic Medicine Athens University School of Medicine Athens Greece) Pharmacokinetics of amikacin in infants and pre school children *Acta Paediatr Scand* 68 419 1979 —The pharmacokinetic properties of amikacin sulfate in infants and children aged from three weeks to 6 years were studied during treatment with doses of 7.5 mg/kg every 12 hours using standard assay methods and technique of two compartment open model kinetic analysis. Peak serum concentrations of amikacin were measured 30 or 60 min after the first intramuscular injection. These ranged from 11.8 µg/ml to 23 µg/ml in infants and from 9.0 µg/ml to 29 µg/ml in children. Five minutes after the first intravenous bolus injection they varied from 16 µg/ml to 29.8 µg/ml in infants and from 34 µg/ml to 42 µg/ml in children. Twelve hours after injection serum concentrations were less than 0.8 µg/ml in all patients. Mean serum half lives of amikacin in infants and children were 2.1 hours and 2.0 hours after intramuscular and 2.2 and 2.0 hours after intravenous administration respectively. No evidence of accumulation was observed after four days treatment. The amount of antibiotic recovered within 12 hours from the urine in all patients ranged from 34.5 to 65% of an intramuscular dose and from 45.8 to 63.3% of an intravenous dose. The dosage regime of 7.5 mg/kg body weight given every 12 hours should be safe and effective for the treatment of infections in the age groups studied.

KEY WORDS Amikacin pharmacokinetics half life urinary excretion infants and pre-school children

During the last two years an increasing number of infections caused by gentamicin resistant gram negative bacteria occurred in the Aglaia Kynakou Children's Hospital Athens. Amikacin a new semisynthetic aminoglycoside antibiotic which is stable to most gentamicin inactivating enzymes has proved useful in the treatment of such infections. Its use is therefore increasing in our hospital and since similar experience of increasing bacterial resistance to gentamicin has been reported by others (3) a more extensive usage of amikacin is generally expected. The kinetics of amikacin have been extensively studied in adults and neonates (1, 2, 4) but there is little information about children aged from two weeks to 6 years (7, 9). Infections in the latter age group are often due to gram negative bacteria (infected

burns and accidental wounds peritonitis chest infections in cystic fibrosis compromised defense systems etc.) and if gentamicin resistant are likely to be treated with amikacin.

We have therefore undertaken a pharmacokinetic study of amikacin in infants and children aged from three weeks to 6 years.

PATIENTS AND METHODS

Twenty four infants and children all inpatients at the Aglaia Kynakou Children's Hospital were studied. They all suffered from serious infections for which amikacin was indicated. The diagnosis and the clinical response are shown in Table 1.

These 4 patients were aged 70 days to 6 years. There were 13 infants of a mean age of 3.5 months and 11 children of a mean age of 3.1 years. The dosage of amikacin sulfate was 7.5 mg/kg body weight given every 12 hours.

- serum concentrations in the premature infant during the first month of life (abstract) *Acta Endocrinol (Abh)* Suppl 215 1978 p 71
- 15 Jacobsen B B Andersen H J Dige Petersen B & Hummer L Pituitary thyroid responsiveness to thyrotropin releasing hormone in preterm and small for gestational age newborns *Acta Paediatr Scand* 66 541 1977
 - 16 Jacobsen B B Andersen H J Petersen B Dige Petersen H & Hummer I Serum levels of thyrotropin, thyroxine and triiodothyronine in fullterm, small for gestational age and preterm newborn babies *Acta Paediatr Scand* 66 681 1977
 - 17 Jacobsen B B Petersen B Andersen H J & Hummer L Serum concentrations of thyroxine binding globulin, prealbumin and albumin in healthy fullterm, small for gestational age and preterm newborn infants *Acta Paediatr Scand* 1979 (accepted for publication)
 - 18 Jacobsen B B Petersen B & Hummer L Serum concentrations of thyrotropin, thyroid hormones and thyroid hormone binding proteins during acute and recovery stages of idiopathic respiratory distress syndrome *Acta Paediatr Scand* 68 257 1979
 - 19 Koivisto M Simila S Haapalahti J & Reimila M Thyroid function assay using the effective thyroxine ratio in infancy *Scand J Clin Lab Invest* 33 195 1974
 - 20 Krasilnikoff P A *Albumin metabolism in normal, mature and premature children* FADL Copenhagen 1975
 - 21 Montalvo J M Wihner H W Mayberry W E & Lum R K Serum triiodothyronine, total thyroxine and thyroxine to triiodothyronine ratios in paired maternal-cord sera and at one week and one month of age *Pediatr Res* 7 706 1977
 - 22 O'Halloran M T & Webster H L Thyroid function assays in infants *J Pediatr* 91 916 1977
 - 23 Robbins J Thyroxine binding proteins. In G A Jameson & T J Greenwalt (eds) *Progress in clinical and biological research*. Elsevier, Oxford 1976 p 331
 - 24 Ryness J The measurement of serum thyroxine in children *J Clin Pathol* 25 776 1977
 - 25 Siegel S *Nonparametric statistics for the behavioral sciences* McGraw Hill Kogakusha Tokyo 1976

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These 4 patients were aged 70 days to 6 years. There were 13 infants of a mean age of 3.5 months and 11 children of a mean age of 3.1 years. The dosage of amikacin sulfate was 7.5 mg/kg body weight given every 12 hours.

Table 1 Clinical condition and response to treatment of the 24 patients studied

M male F female IM intramuscular IV intravenous

Diagnosis	No of patients		Route of administ		Response to treatment		
	M	F	IM	IV	Cured	Improved	Failed
Urinary tract infection	4	5	9	-	8	1	-
Peritonitis	6	1	6	1	5	1	1
Infected burn	5	-	2	3	4	1	-
Septicemia	2	-	-	2	2	-	-
Pneumonia	1	-	1	-	1	-	-
Wound infection	1	-	1	-	1	-	-

The antibiotic was administered by intramuscular (i m) injection in 18 patients of whom 10 infants (7 male and 3 female) and 8 children (6 male and 2 female) and 15 bolus intravenous (i v) injection in 6 three infants (all male) and 3 children (2 male and one female). If patients required an additional antibiotic amikacin was given alone for the first 12 hours of therapy and then the second drug was added. Blood samples were obtained by heel prick or venipuncture prior to and at 1/2 1 2 4 6 8 and 12 hours after the first dose. Urine was collected in plastic bags before and at 0-2 2-4 4-8 and 8-12 hours post dose. To detect possible accumulation of the drug blood specimens were also obtained prior to the morning injection on the fourth treatment day.

Concentrations of amikacin were measured using an agar well plate technique with *Bacillus subtilis* 1904T as indicator organism. In blood samples from patients treated with amikacin plus either lincomycin or clindamycin a strain of *Klebsiella* was used as indicator organism and for specimens from those receiving also penicillin G or ampicillin penicillinase concentrate BBL 150 was added. Large plates were used and standards (ranging from

10 µg/ml to 700 µg/ml) as well as specimens suitably diluted when necessary were set in triplicate. Serum half life was determined by using a two compartment open model as follows: after the distribution phase was completed the equation for the regression line of the log of serum antibiotic concentrations was calculated by the method of the least mean squares. The half life was determined by dividing the $\log_{10} 2$ by the slope of the line.

RESULTS

Mean serum concentrations of amikacin in infants and children following the first i m or i v injection are shown in Table 2.

In 10 infants receiving i m injections peak serum concentrations were obtained 30 to 60 min post dose and ranged from 11.8 µg/ml to 23.0 µg/ml (mean 18.4 ± 3.0). Four hours post dose levels averaged 4.2 µg/ml and at 8 hours

Table 2 Mean serum concentration of amikacin in infants and pre school children after i m or i v injection of 7.5 mg/kg

Age group and route	No of patients	Serum concentration (µg/ml) at indicated times (minutes or hours after dose)					
		5	30	1	2	4	8
Infants i m	10		17 ± 3.6 (11.8-23.0)	15.7 ± 2.8 (9.0-19.5)	10.6 ± 3.4 (3.2-16.4)	4.3 ± 1.1 (1.7-5.9)	2.6 ± 0.34 (2.2-3.7)
Children i m	8		18.4 ± 5.4 (8.0-29.0)	17.7 ± 3.7 (9.0-22.0)	11.6 ± 4.6 (2.8-17.5)	3.7 ± 1.4 (1.5-5.9)	2.2 ± 1.0 (0.8-3.7)
Infants i v	3	24.3 ± 5.9 (16.0-29.8)	14.7 ± 4.8 (7.9-18.4)	11.6 ± 5.1 (4.4-16.2)	8.7 ± 4.8 (2.4-14.0)	5.1 ± 3.1 (1.2-8.9)	3.8 ± 0.4 (3.4-4.7)
Children i v	3	37.3 ± 3.4 (34.0-42.0)	19.8 ± 3.2 (16.4-24.1)	13.1 ± 2.3 (9.4-16.0)	7.8 ± 0.5 (7.1-8.2)	4.0 ± 0.1 (3.9-4.2)	2.0 ± 0.3 (1.6-2.4)

Figures with a \pm sign indicate standard deviation in parenthesis are ranges

* Only two specimens studied

Table 3 Mean urinary excretion of amikacin after *i m* or *i v* injection of 7.5 mg/kg in infants and children

NUP: no urine passed in this collection period

Age group and route	No of pat	Mean urinary concentration ($\mu\text{g/ml}$ first column) and mean percentage recovered (second column) at indicated collection periods (hours)				
		0-2	2-4	4-8	8-12	0-12
Infants <i>i m</i>	8	61 16.3 \pm 1.7 (NUP-31.1)	336 14.3 \pm 8.7 (6.2-30.0)	157 6.6 \pm 2.6 (4.4-11.0)	31 1.8 \pm 0.8 (0.8-3.9)	39.0 \pm 5.1 (34.5-48.7)
Children <i>i m</i>	3	345 18.5 \pm 13.0 (NUP-28.8)	215 10.2 \pm 7.2 (NUP-15.8)	184 19.7 \pm 10.4 (6.7-31.5)	134 6.3 \pm 7.0 (NUP-16.7)	54.6 \pm 7.4 (47.7-65.0)
Infants <i>i v</i>	3	561 4.7 \pm 11.0 (9.1-37.8)	51 3.3 \pm 15.0 (9.3-43.3)	78 7.8 \pm 7.1 (NUP-5.3)	31 3.2 \pm 2.2 (1.1-6.4)	53.0 \pm 5.4 (45.8-58.9)
Children <i>i v</i>	3	64 24.0 \pm 1.8 (5.9-34.3)	490 20.4 \pm 6.4 (15.4-29.5)	168 15.1 \pm 5.7 (10.8-27.5)	74 2.0 \pm 0.8 (0.8-3.9)	61.5 \pm 7.0 (58.7-63.7)

Figures with a \pm sign indicate standard deviation in parenthesis are ranges1.5 $\mu\text{g/ml}$ with a mean serum half life of 2.1 hours

In 3 infants treated with *i v* injections peak serum concentrations were measured 5 min post dose and ranged from 16.0 $\mu\text{g/ml}$ to 29.8 $\mu\text{g/ml}$ (mean 24.3 ± 5.9). Thirty minutes after injection serum levels averaged 14.7 $\mu\text{g/ml}$ at 4 hours 5.1 $\mu\text{g/ml}$ and at 8 hours they averaged 1.4 $\mu\text{g/ml}$. Mean serum half life was 2.2 hours in these patients.

In 8 of the older children who received *i m* injections peak serum concentrations were achieved from 30 to 60 min after the dose and

ranged from 9.0 $\mu\text{g/ml}$ to 29.0 $\mu\text{g/ml}$ (mean 19.3 ± 5.1). Four hours after injection serum levels averaged 3.7 $\mu\text{g/ml}$ and at 8 hours 1.3 $\mu\text{g/ml}$ while serum half life averaged 2.0 hours.

Finally in 3 children receiving *i v* injections peak serum levels 5 min after injection ranged from 34.0 $\mu\text{g/ml}$ to 42.0 $\mu\text{g/ml}$ (mean 37.3 ± 3.4). Thirty minutes post dose mean serum levels were 19.8 $\mu\text{g/ml}$ while at 4 hours levels averaged 4.0 $\mu\text{g/ml}$ and at 8 hours 1.0 $\mu\text{g/ml}$. Serum half life in these children averaged 2.0 hours.

In all patients amikacin blood levels were undetectable (less than 0.8 $\mu\text{g/ml}$) 12 hours post dose. No cumulation of the drug was observed on the fourth treatment day serum levels before the morning injection being undetectable in all but one patient a 5 month old girl in whom blood levels was 1.1 $\mu\text{g/ml}$.

Excretion of the drug in urine of infants and children is shown in Table 3. In 8 infants 34.5% to 48.7% of the *i m* dose was recovered within 12 hours. After *i v* injection in 3 infants 45.8% to 58.9% of the drug was recovered during the same period.

In 3 children aged from one to 8 years recovery rate ranged from 47.7% to 65% of the *i m* dose and in another 3 children given *i v* injections it ranged from 58.7% to 63.2%.

		Mean serum half life (hours)
1.5	<0.8	15.0 \pm 0.17 (1.9-40)
3.0	<0.8	0.0 \pm 0.74 (1.6-2.78)
4.0	<0.8	7.7 \pm 0.15 (1.0-33)
0.0	<0.8	0.4 \pm 0 (1.79-30)

Urine levels of amikacin were much higher after i.v. injection ranging in the first four hours from 186 $\mu\text{g/ml}$ to 750 $\mu\text{g/ml}$ in infants and from 210 $\mu\text{g/ml}$ to 806 $\mu\text{g/ml}$ in children. After i.m. injection it ranged from 40 $\mu\text{g/ml}$ to 950 $\mu\text{g/ml}$ in infants and from 110 $\mu\text{g/ml}$ to 495 $\mu\text{g/ml}$ in children. Urine concentrations were bactericidal in all patient groups for the whole period of 12 hours after injection.

DISCUSSION

Pediatric drug therapy and particularly calculation of dosage is complicated by the rapid change in body weight and body composition in children (8).

Studies with amikacin sulfate in adults have shown that the desirable peak serum concentrations are 15–35 $\mu\text{g/ml}$ and 5.0 $\mu\text{g/ml}$ or less at the time of the next dose (5). It has also been shown that the serum half life of aminoglycosides is approximately two hours in persons with normal renal function and excretion is mainly affected by glomerular filtration (2, 5).

This study involving 24 seriously ill infants and children has shown that mean peak blood concentrations of amikacin given intramuscularly or intravenously in a dose of 7.5 mg/kg every 12 hours were in the therapeutic and safe range for both age and routes of administration. On the other hand 8 hours following administration blood concentrations were already below the therapeutic levels for many pathogens. However all patients included in the study were clinically and bacteriologically cured (Table 1) (6). Serum half life measured at the phase of elimination was found to be almost the same as that reported for adults (2, 5) but it was slightly longer in infants (about 2.2 hours) than in children (about 2.0 hours). Vogelstein and his colleagues (9) using a new pharmacokinetic technique have reported somewhat shorter half lives (1.6 hours) in children aged from 4 to 15 years and reported no

cumulation after four 8 hourly doses of 470 mg/m. Whether continuation of an 8 hourly regime would result in eventual cumulation and potential toxicity requires further investigation. However at least for the age groups that we have investigated that is 20 days to 6 years such high dosage and frequent administration of amikacin may not be necessary in view of our satisfactory results with 7.5 mg/kg given every 12 hours (Table 1).

REFERENCES

- 1 Cabani B E & Taggart J G. Comparative pharmacokinetics of BBK-8 and kanamycin in dogs and humans. *Antimicrob Agents Chemother* 3:418 1973.
- 2 Clarke J T, Libke R D, Regamey C & Kirby W M. Comparative pharmacokinetics of amikacin and kanamycin. *Clin Pharmacol Ther* 15:610 1974.
- 3 George M H & Healing D E. Gentamicin resistance. *Lancet* i 259 1976.
- 4 Howard J B, McCracken G H, Trujillo H & Mohs E. Amikacin in newborn infants: comparative pharmacology with kanamycin and clinical efficacy in 45 neonates with bacterial diseases. *Antimicrob Agents Chemother* 10:205 1976.
- 5 Jackson G G. Present status of aminoglycoside antibiotics and their safe effective use. *Clin Therapeut* 1:200 1977.
- 6 Kafetzis D A, Kosmidis J C, Sinaniotis C A & Papadatos C J. Therapeutic and kinetic studies with amikacin in infants and children. In W. Siegenthaler & R. Luthy (eds) *Current Chemotherapy*. American Society for Microbiology, Washington DC 1979, vol. 2, p. 1002.
- 7 Khan A J, Evans H E, Jhaveri R, Chang C T & Hochstein L. Amikacin pharmacokinetics in the therapy of childhood urinary tract infection. *Pediatrics* 58:773 1976.
- 8 Rane A & Wilson J T. Clinical pharmacokinetics in infants and children. *Clin Pharmacokin* 1:2 1976.
- 9 Vogelstein B, Kowarski A A & Lietman P S. The pharmacokinetics of amikacin in children. *J Pediatr* 91:333 1977.

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DOSAGE OF SALICYLATES FOR CHILDREN WITH JUVENILE RHEUMATOID ARTHRITIS

A Prospective Clinical Trial with Three Different Preparations of Acetylsalicylic Acid

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ABSTRACT Makela A L Yrjana T and Mattila M (Departments of Paediatrics and Clinical Chemistry University of Turku and Department of Pharmacology University of Helsinki Finland) Dosage of salicylates for children with juvenile rheumatoid arthritis Acta Paediatr Scand 68 423 1979—41 children with juvenile rheumatoid arthritis (JRA) and 6 with postinfectious arthropathies aged 3-15 years were treated with acetylsalicylic acid for 14 days during which time the patients were hospitalized Three different acetylsalicylic acid preparations were used a microencapsulated form an enteric coated form and standard acetylsalicylic acid tablets Serum salicylate concentrations were measured by Trinder's photometric method With doses of 90-120 mg/kg/day symptoms of salicylism appeared in about 50% of the cases Daily doses of 2 g/m² (not exceeding 70 mg/kg) proved relatively safe in this study whereas symptoms and signs of intoxication appeared at doses exceeding 3 g/m²/day In this respect there were no significant differences between the three acetylsalicylic acid preparations used The results of this study also suggest that the serum salicylate concentrations should not exceed 2000 µmol/l (about 27 mg/100 ml) The symptoms of salicylism correlated closely with serum salicylate levels which in turn correlated well with the dosage in g/m² Elevation of serum aspartate aminotransferase was noted in 1/3 of the cases All of these had a dose exceeding 2 g/m² and the frequency of elevated enzyme activities increased with increasing dosage In the group receiving enteric-coated form of acetylsalicylic acid there were fewer positive benzidine tests (12%) than in the two other groups (22-28%)

KEY WORDS Salicylates juvenile rheumatoid arthritis aspartate aminotransferase

In order to achieve an anti-inflammatory effect of acetylsalicylic acid (ASA) doses approaching toxic ones may be required (1-7). However the dose-dependent pharmacokinetics of salicylate make the dosage of ASA problematic particularly in children. At high serum levels the elimination of salicylate is very slow and this will easily lead to accumulation of the drug. Not all authors have accepted the necessity for the high dosage of salicylates (2, 10, 16, 39) and numerous warnings have been published concerning the toxicity of ASA both in adults and in children (11, 14, 15, 24).

In children with juvenile rheumatoid arthritis (JRA) the daily dosage of ASA recommended in modern textbooks is 90-100 mg/kg

or even higher (3, 4, 12, 34, 35, 36, 38, 40, 43). When related to the body surface area the recommended daily dose is 3.0 g/m² (8).

Our preliminary results (17, 27, 28, 29, 30) which are in agreement with earlier warnings suggest that the widely accepted high dosage recommendations may be incorrect as the risk of salicylate intoxication seems to be very high. Therefore we conducted a prospective study to assess the limits of safe ASA dosages for children.

PATIENTS AND METHODS

47 children aged 3-15 years were divided into three groups: 17 children received ASA in enteric-coated tablets (Prunaspas® Läke OY), 11 children received ASA in micro-

Table 1 Age and sex of the patients in different treatment groups

Treatment	3-5 years		6-10 years		11-15 years		Total
	♀	♂	♀	♂	♀	♂	
Enteric coated ASA Primaspan [®]	1	-	3	7	5	1	17
Microencapsulated ASA Medisyl [®]	4	1	5	3	4	4	21
Aspirin [®] Bayer	-	-	6	-	3	-	9
Total							47

encapsulated tablets (Medisyl[®] Medica) and 9 children received standard uncoated ASA tablets (Aspirin[®] Bayer). The age and sex of the children in these three groups are shown in Table 1.

ASA was administered for 14 days during which time the patients remained in hospital for close observation. All children entered the study with the permission of their parents.

The daily dosage of ASA was divided in three doses administered at 8 a.m., 2 p.m. and at 9 p.m.

41 patients suffered from JRA and 6 patients from post-infectious arthropathies. None of them had proteinuria. Other non-steroidal anti-inflammatory agents were withdrawn before the trial. During the test six patients continued their maintenance gold therapy and 12 patients their therapy with hydroxychloroquine. Six patients received prednisolone during the trial (five of these intermittently (5-15 mg as single dose every other morning) and one continuously (7.6 mg every morning)).

Before and during salicylate treatment the following laboratory tests were performed at weekly intervals: acid-base balance, ESR (erythrocyte sedimentation rate), Hb (haemoglobin), WBC (white blood cell count), thrombocytes, S-ASAT (serum aspartate aminotransferase), bleeding time and P+P prothrombin test (thromboplastin time, Simplastin A[®]).

The levels of S-ASAT accepted as normal (33) were <45 U/l in patients aged 2-9 years and <35 U/l in those over 9 years of age. Bleeding time <5 min was considered as normal as well as a value of P+P prothrombin test between 0.70-1.30.

The possible presence of occult blood in stools was checked daily with the benzidine test. The Addis count test for erythrocytes and leukocytes in urine was done twice a week. An erythrocyte excretion rate of more than 1000 erythrocytes/min and a leukocyte excretion rate of more than 2000 leukocytes/min were considered pathological.

Serum salicylate levels were determined by Snyder's photometric method (42). The precision of the method is 14.9 µmol/l (S.D.) at the level 1000 µmol/l (n=109). The blood samples were drawn every morning at 8 a.m. before the first daily dose of ASA. On the first fifth and tenth day of medication additional samples were collected at noon and at 6.30 p.m.

RESULTS

The mean daily dose of ASA in the whole group of 47 patients was 89 ± 15 mg/kg (S.D.) (range 52-117 mg/kg). When calculated in square meters of body surface area the mean daily dose was 2.4 ± 0.5 g/m² (range 1.4-3.7 g/m²).

The first symptoms of salicylism appeared on the fifth day of therapy. 19 out of 47 patients (40.5%) developed toxic symptoms and their treatment was therefore discontinued after a medication period varying from 9 to 13 days.

The syndrome of salicylism was mainly manifested by headache, tinnitus, dizziness, difficulty in hearing, impaired vision, sweating, nausea, vomiting, nasal congestion and slight hyperpyrexia. In five patients all over 12 years old and weighing more than 40 kg a more severe salicylate intoxication was characterized by hyperventilation, mental confusion, lassitude, restlessness, irritability, hyperthermia and disturbances in acid-base balance, mainly respiratory alkalosis. The treatment was discontinued on the 6th-7th day in these cases. In one girl aged 13 years hepatotoxicity and encephalopathy (Reye's syndrome) was encountered. This began with

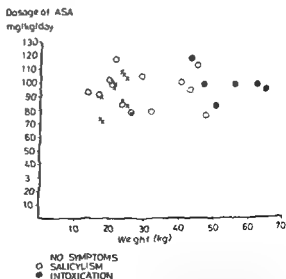


Fig. 1 Relation of the body weights of the patients and the daily dosage of ASA in mg/kg to the toxic symptoms.

Table 2 Relation of the daily dosage of ASA (mg/kg and g/m²) to the development of symptoms of salicylism and to increased levels of S ASAT

Daily dose of ASA	No of patients	Symptoms of salicylism No (%)	Increased S ASAT No (%)
≤70 mg/kg	7	— (0)	(*)8
71-90 mg/kg	15	5 (33)	7 (47)
91-110 mg/kg	5	14 (56)	6 (4)
<1 g/m ²	7	— (0)	— (0)
1-2 g/m ²	35	14 (40)	12 (34)
≥3 g/m ²	5	5 (100)	3 (60)

subfebrile temperature and progressed to mental confusion restlessness hyperventilation hyperpyrexia haematemesis vertigo convulsions and coma. The symptoms appeared on the 10th day of treatment with a daily dose of 87 mg/kg (2.64 g/m²). This case is described in detail elsewhere (37).

Symptoms of salicylism or severe salicylate intoxication were observed in all children weighing more than 40 kg if the dosage of ASA was 80 mg/kg/day or higher (Fig. 1).

In Table 2 the daily dosages of ASA are related to toxic symptoms.

Serum salicylate levels related well to the symptoms of salicylism (Table 3). In each individual case the mean salicylate serum level was calculated from the morning values obtained during the period of steady state. If steady state was not reached the last morning value before the discontinuation of medication was used.

The daily dosage of ASA calculated in g/m² correlated well with the salicylate serum levels (Fig. 2) whereas the dosage in mg/kg did not (Fig. 3). Different salicylate preparations used in these tests did not show any differences in this respect. According to these results the therapeutic serum salicylate levels should not exceed 2000 µmol/l (about 27 mg/100 ml).

Acid-base balance disturbances during salicylate therapy manifested themselves mainly as depletion of the arterial P_{CO}₂ and standard bicarbonate. Also patients without any clinical

Table 3 Salicylate serum levels related to the symptoms of salicylism

Salicylate serum levels	No of patients	Salicylism No (%)
<100 µmol/l (<15 mg/100 ml)	8	— (0)
100-1600 µmol/l (15-27 mg/100 ml)	8	2 (5)
1610-2300 µmol/l (27-37 mg/100 ml)	19	11 (31)
2310-2900 µmol/l (37-40 mg/100 ml)	7	6 (86)
2910-3600 µmol/l (40-50 mg/100 ml)	5	5 (100)
Total	47	19

symptoms of salicylism showed depletion of P_{CO}₂ to 2.95-3.9 kPa in 14 cases and standard bicarbonate to 20 mmol/l or less in six cases.

In children displaying no clinical symptoms of salicylism (28 patients) drug administration continued for a full fortnight. Steady state in salicylate serum levels was reached on the 3rd to 7th day of treatment. In line with the fact that the apparent half life of salicylate elimination increases with increasing dose (18-32) the time interval needed to reach steady state in salicylate serum levels became longer the higher the fixed daily dose was. With doses as high as 2.9 g/m²/day steady state was not achieved until the 7th or 8th day from the start of the treatment.

Fig. 4 presents mean serum salicylate levels obtained during the two weeks of treatment with the three different types of ASA preparations. The mean dosage in the three groups of patients was virtually identical. At the beginning of the treatment the serum levels rose more slowly in the Primaspan group reflecting the slow release character of this enteric coated tablet (41). During steady state there were no significant differences between salicylate serum levels in morning samples in the different groups. A tendency towards decreasing serum salicylate levels was found after 5 days which is in agreement with the findings by Muller et al. (26).

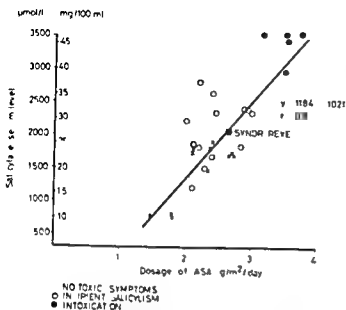


Fig. 2 Correlation of salicylate serum levels with the daily dosage of ASA per m^2 of body surface area.

Elevation of the serum aspartate aminotransferase activity (S ASAT) from normal to pathological values was noted in 12 girls and 3 boys (32%). The abnormal values remained below 100 U/l except in 3 cases in whom the respective peak values were 203, 252 and in the patient with toxic encephalopathy 2535 U/l. Symptoms of salicylism were observed in five of these 15 cases and apparent salicylate intoxication in two. No eosinophilia or other features suggestive of drug allergy could be detected in these patients.

Elevated values of S ASAT were noted during the first week of treatment in 4 cases (on the 5th or 6th day). Two of these patients had symptoms of salicylism and two displayed obvious signs of salicylate intoxication. In all these cases salicylate therapy was discontinued after the first week. In eleven other patients the elevated values of S ASAT were noted after 10 to 14 days from the start of the salicylate treatment.

Elevated values of S ASAT returned to normal usually within one week after discontinuation of salicylate treatment. Subsequently it was possible to resume ASA medication at reduced dosage without increase in enzyme activity.

All but one of the patients with elevated S ASAT values had serum salicylate levels $\geq 1810 \mu\text{mol/l}$ ($\geq 25 \text{ mg/100 ml}$). The frequency of elevated S ASAT values increased with increasing dosage of ASA (Table 2) the lowest daily dose accompanied by pathological values of S ASAT being 2 g/m. On the other hand there was no strict correlation between the occurrence of elevated S ASAT activity and the development of salicylism nor did elevated values of S ASAT show any correlation with the clinical activity of rheumatoid arthritis or ESR.

In Table 4 side effects are listed separately for each group of patients receiving a particular salicylate preparation.

Benzidine tests for blood in faeces were occasionally positive in ten cases. All but one changed to negative at subsequent controls despite continued salicylate treatment. No decrease in haemoglobin values was observed in these patients.

Positive benzidine tests were less frequent in the group receiving the enteric coated tablets of ASA than in the others. Otherwise the side effects were similar in all three groups.

Haematuria and leukocyturia occurred in 21–25% of the cases. The number of leuko-

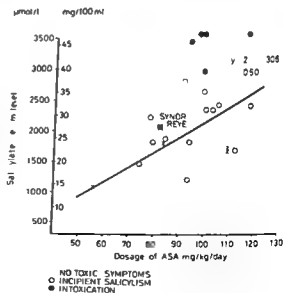


Fig. 3 Correlation of salicylate serum levels with the daily dosage of ASA per kg of body weight.

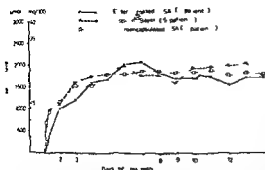


Fig 4 Mean salicylate serum levels with different ASA preparations with approx identical daily dosage: x—x Enteric coated ASA (Primaspan®) 5 patients Mean daily dose 90 ± 9.6 mg/kg (4 ± 0.6 g/m²) ●—● Uncoated ASA tablets (Aspirin®) 5 patients Mean daily dose 82 ± 15.3 mg/kg (3.5 ± 0.7 g/m²) □—□ Microencapsulated ASA (Medisyl®) 7 patients Mean daily dose 93 ± 16 mg/kg (4 ± 0.7 g/m²)

cytes was rising to 10 000 cells/min or more in 3 cases and that of erythrocytes to more than 3 500 cells/min in two cases

Prolonged bleeding time or pathological values in P+P prothrombin test did not relate to clinical symptoms in this study. All five patients showing pathological values in the P+P prothrombin test (the values being between 0.19–0.65) had symptoms of salicylism; two of them also increased values of S ASAT.

DISCUSSION

Salicylic acid is eliminated by urinary excretion of the unchanged compound (glomerular filtration with passive tubular reabsorption) and by metabolic conversion to salicyluric acid (conjugation with glycine), salicyl phenolic glucuronide, salicyl acyl glucuronide and gentisic acid (44). The kinetics of salicylic acid are dose-dependent because the formation of salicyluric acid and salicyl phenolic glucuronide becomes saturated already at therapeutic dosage (18, 22, 23, 32). According to Levy (18) the relationship between the dose and the time required to eliminate half the salicylate amount is as follows: (a) When the dose is so small that all elimination processes follow

first-order kinetics the half life is about 2.9 h. (b) When the dose is so large that the contribution of the salicyluric acid formation process to the elimination of salicylate is negligible the half life increases to about 22 h. At such high dosage elimination occurs mainly by salicylate excretion and salicylglucuronide formation. (c) When the administered dose is of intermediate size the formation of salicyluric acid can significantly contribute to the total elimination of salicylate. Considerable inter- and intrasubject variations can be expected in maximum half lives of salicylate elimination even up to 41 h. The results of the present study are in accordance with this concept.

The renal clearance of salicylic acid is extremely sensitive to urinary pH (21, 22). Administration of antacids with aspirin can cause an appreciable reduction of serum salicylate levels (20). This fact may offer at least a partial explanation of the differences between the results reported (5, 6, 17, 30).

The long metabolic half life of salicylates in high serum levels permits more widely spaced administration of the drug per 24 h. In the present study a tid dosage regimen was chosen instead of a four or five times daily regimen to avoid awakening of the children at midnight. Our clinical experience is in accordance with recent data from Levy et al (19) that at sufficiently high daily dosage of ASA toxic symptoms cannot be avoided by dividing the total daily dose of ASA into smaller increments given at shorter intervals.

According to the results of this study a daily dosage of ASA 2 g/m² (not more than 70 mg/kg) has proved to be relatively safe. It is important to avoid loading the patients with heavy doses of ASA which exceed the elimination capacity of the body.

An inappropriate method of calculating the dose may contribute to the appearance of salicylate toxicity. For example a modern textbook recommends that in order to achieve a blood salicylate level of 20 mg/100 ml (1450 µmol/l) a dose of 3 g/m²/24 h is adequate (31). This statement seems to be incorrect or at

Table 4 Frequency of side effects in different groups

	Enteric coated ASA Primaspan®	Microcrystall ASA MEDISYL®	Aspirin® Bayer	Total
Number of patients	17	21	9	47
Dosage of ASA mg/kg/day (mean \pm S.D.)	87 \pm 16.8	90 \pm 15	89.5 \pm 13	89 \pm 15
Dosage of ASA g/m ² /day (mean \pm S.D.)	2.4 \pm 0.5	2.5 \pm 0.6	2.4 \pm 0.2	2.4 \pm 0.5
Faecal blood loss				
No	2	6	2	10
%	12	28	22	21
Haematuria >1000 ER/min				
No	4	6	-	10
%	24	28	-	21
Leukocyturia >2000 Leuk/min				
No	4	8	1	13
%	24	38	11	25
Elevated S-ASAT				
No	5	7	3	15
%	29	33	33	32
Pitrol P+P prothrombin test				
No	3	2	-	5
%	17.5	9.5	-	10.5
Prolonged bleeding time				
No	3	3	1	7
%	17.5	14	11	15
Salicylism				
No	8	4	2	14
%	47	19	22	40
Intoxication				
No	2	3	-	5
%	12	14	-	10
Hepatotoxicity and encephalopathy				
No	-	1	-	1
%	-	4	-	2

least misleading. With a constant daily dose of 3 g/m of unbuffered ASA the serum levels achieved during steady state are much higher i.e. about 35 mg/100 ml (2540 μ mol/l) (Fig. 2) and are very likely to lead to intoxication.

In this study the elevations of enzyme activity of serum aspartate aminotransferase were related to high salicylate serum levels though not necessarily with symptoms of salicylate intoxication. The 'threshold' toxic salicylate dosage in the present study appeared to be 2 g/m² of body surface area per day. Calculation of the dose in mg per kg of body weight is less satisfactory than per square meter of body surface. None of the patients in-

cluded in the present study showed any liver function disturbances before the tests. Among the patients who reacted with an elevated S-ASAT were also 4 children with post infectious arthropathies. Thus the possible systemic manifestation of JRA did not seem to be the primary cause of liver damage. Moreover it is still the subject of debate whether a moderate rise in transaminases reflects hepatic toxicity or a change in enzyme turnover. The transient nature of the S-ASAT elevation during salicylate treatment suggests also that the toxic effects of salicylates are relatively benign but also that transaminase values should be monitored in all children receiving long term sali-

cyate therapy (13-25) Wolfe and coworkers (45) have pointed out that the continuation of ASA therapy after the onset of hepatic dysfunction can result in more severe liver disease. The case of hepatotoxicity and encephalopathy might be an example of this possibility (37).

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REFERENCES

- Boardman P L & Hart F D. Clinical measurement of the antiinflammatory effects of salicylates in rheumatoid arthritis. *Br Med J* 4: 464 1967.
- Bywaters E G L. The treatment of rheumatic fever. *Trans Med Soc Lond* 72: 06 19 66.
- Calabro J J. Juvenile rheumatoid arthritis. In L J Hollander & D J McCarty Jr (eds). *Arthritis and allied conditions*. Lea & Febiger, Philadelphia 1973, 8th ed. p 399.
- Calabro J J. Diseases of connective tissue. Juvenile rheumatoid arthritis. In S S Gellis & B M Kagan (eds). *Current pediatric therapy*. 6th ed. Saunders Company, Philadelphia 1973, p 377.
- Calabro J J. Dosage of salicylates for children with juvenile rheumatoid arthritis. In S S Gellis (ed). *Year book of pediatrics*. Year Book Medical Publishers, Chicago 1977, p 384.
- Calabro J J, Burnstein S L & Staley H L. Dosage of salicylates for children with juvenile rheumatoid arthritis. Letter to the editor. *Scand J Rheumatol* 5: 751 1976.
- Calabro J J & Paulus H E. Anti-inflammatory effect of acetylsalicylic acid in rheumatoid arthritis. *Clin Orthop* 71: 14 1970.
- Fanconi G & Walligen A. *Lehrbuch der Paedatrie*. Benno Schwabe & Co. Verlag, Basel and Stuttgart 1946, p 94.
- Fashen G L & Walker J N. Salicylate intoxication. Studies on the effects of sodium salicylate on prothrombin time and alkali reserve. *Am J Dis Child* 68: 396 1944.
- Fischel E E. The use of salicylate in rheumatic fever. *Bull Rheum Dis* 2: 9 1951.
- Graham J D P & Parker W A. The toxic manifestations of sodium salicylate therapy. *Q J Med* 17: 153 1949.
- on Harnack H A. *A neumitteldosierung im Kindesalter*. Voraussetzungen, Methoden und Ergebnisse systematischer Dosierungsuntersuchungen. Georg Thieme Verlag, Stuttgart 1965, p 40.
- Hinkel G K, North G & Lorenz K. Salicylat bedingte toxische Hepatosen bei Kindern. *Das deutsche Gesundheitswesen* 32: 164 1977.
- Holt K S. Salicylates in rheumatic fever. Difficulties experienced in treating children with large doses. *Lancet* II 1197 1954.
- Jager H V & Alway H. The treatment of acute rheumatic fever with large doses of sodium salicylate. With special reference to dose management and toxic manifestations. *Am J Med Sci* 211: 773 1946.
- Keth J D & Ross A. Observations on salicylate therapy in rheumatic fever. *Can Med Assoc J* 52: 554 1945.
- Laaksonen A L & Yrjana T. Die Dosierung der Salicylatprparate bei juveniler Rheumatothritus. *Kindergesundh Prax* 43: 763 1975.
- Levy G. Pharmacokinetics of salicylate elimination in man. *J Pharm Sci* 54: 959 1965.
- Levy G & Giacomini K M. Rational aspirin dosage regimens. *Clin Pharmacol Ther* 23: 247 1978.
- Levy G, Lampman T, Kamath H L & Garrettson L K. Decreased serum salicylate concentrations in children with rheumatic fever treated with antacid. *N Engl J Med* 293: 374 1975.
- Levy G & Leonards J R. Urine pH and salicylate therapy. *JAMA* 217: 81 1971.
- Levy G & Tsuchiya T. Salicylate accumulation kinetics in man. *N Engl J Med* 287: 430 1972.
- Levy G, Tsuchiya T & Amsel L P. Limited capacity for salicyl phenolic glucuronide formation and its effect on the kinetics of salicylate elimination in man. *Clin Pharmacol Ther* 13: 758 1973.
- Levy G & Yaffe S J. The study of salicylate pharmacokinetics in intubated infants and children. *Clin Toxicol* 1: 409 1968.
- Miller J J III & Weissman D H. Correlations between transaminase concentrations and serum salicylate concentration in juvenile rheumatoid arthritis. *Arthritis Rheum* 19: 115 1976.
- Muller F O, Hundt H K L & Muller D G. Pharmacokinetic and pharmacodynamic implications of long term administration of non steroidal anti-inflammatory agents. *Int J Clin Pharmacol Biopharm* 15: 397 1977.
- Makela A L. Naproxen in the treatment of juvenile rheumatoid arthritis. Metabolism, safety and efficacy. *Scand J Rheumatol* 6: 193 1977.
- Makela A L & Yrjana T. Salicylatdosering for barn med juvenil reumatoid artni. *Asstra nyt* 12: 574 1974.
- Makela A L & Yrjana T. The toxicity of high dosage salicylate therapy in children. *EULAR/WHO workshop on the care of rheumatic children*. March 21-4/25 Oslo, Norway 1977.
- Makela A L, Yrjana T & Haapasalo J. Dosage of salicylates for children with juvenile rheumatoid arthritis. A preliminary report. *Scand J Rheumatol* 4: 50 1975.
- Vaughan V C & McKay R J. *Nelson textbook of pediatrics*. W B Saunders Company, Philadelphia 1975, 10th ed. p 1719.
- Paulus H E, Siegel M, Mongan M, Okun R & Calabro J J. Variations of serum concentrations and half life of salicylate in patients with rheumatoid arthritis. *Arthritis Rheum* 14: 527 1971.

Table 4 Frequency of side effects in different groups

	Enteric coated ASA Primaspirin®	Microcrystall ASA MEDISYL®	Aspirin® Bayer	Total
Number of patients	17	21	9	47
Dosage of ASA mg/kg/day (mean \pm S.D.)	87 \pm 16.8	90 \pm 15	89.5 \pm 13	89 \pm 15
Dosage of ASA g/m ² /day (mean \pm S.D.)	2.4 \pm 0.5	2.5 \pm 0.6	2.4 \pm 0.2	2.4 \pm 0.4
Faecal blood loss				
No	2	6	2	10
Yes	12	28	22	71
Haematuria >1000 ER/min				
No	4	6	-	10
Yes	23	28	-	71
Leukocyturia >2000 Leuk/min				
No	4	8	1	13
Yes	23	38	11	75
Elevated S-ASAT				
No	5	7	3	15
Yes	29	33	33	95
Pathol P+P prothrombin test				
No	3	2	-	5
Yes	17.5	9.5	-	10.5
Prolonged bleeding time				
No	3	3	1	7
Yes	17.5	14	11	15
Salicylism				
No	8	4	2	14
Yes	47	19	22	70
Intoxication				
No	2	3	-	5
Yes	12	14	-	10
Hepatotoxicity and encephalopathy				
No	-	1	-	1
Yes	-	4	-	2

least misleading. With a constant daily dose of 3 g/m of unbuffered ASA the serum levels achieved during steady state are much higher i.e. about 35 mg/100 ml (2540 μ mol/l) (Fig. 2) and are very likely to lead to intoxication.

In this study the elevations of enzyme activity of serum aspartate aminotransferase were related to high salicylate serum levels though not necessarily with symptoms of salicylate intoxication. The threshold toxic salicylate dosage in the present study appeared to be 2 g/m² of body surface area per day. Calculation of the dose in mg per kg of body weight is less satisfactory than per square meter of body surface. None of the patients in-

cluded in the present study showed any liver function disturbances before the tests. Among the patients who reacted with an elevated S-ASAT were also 4 children with post infectious arthropathies. Thus the possible systemic manifestation of JRA did not seem to be the primary cause of liver damage. Moreover it is still the subject of debate whether a moderate rise in transaminases reflects hepatic toxicity or a change in enzyme turnover. The transient nature of the S-ASAT elevation during salicylate treatment suggests also that the toxic effects of salicylates are relatively benign but also that transaminase values should be monitored in all children receiving long term sal-

CORRELATION BETWEEN AGE AND PLASMA LEVEL/DOSAGE RATIO FOR PHENOBARBITAL IN INFANTS AND CHILDREN

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From the Department of Paediatrics, University School of Medicine, Milan, Italy

ABSTRACT Rossi L. N., Nino M. L. and Principi N. (Department of Paediatrics, University School of Medicine, Milan, Italy). Correlation between age and plasma level/dosage ratio for phenobarbital in infants and children. *Acta Paediatr Scand* 68: 431-434, 1979. — 123 plasma concentration measurements of phenobarbital were obtained from 31 children (2 months – 6 1/2 years old) at steady state conditions. The plasma level/dosage ratio has been found to have a highly significant correlation with the age of the patient both for dosage in mg/kg and in mg/m². The ratio increases with the increase in the age of the patient in a rate which is greater for dosages expressed on the basis of body weight. Moreover, at least for body weight related dosages, this increase is relatively high in the first year of life, becoming less marked after. Practical indications are given about the required dosage of phenobarbital in different groups of ages from 2 months up to 6 1/2 years. It is recommended however to regularly measure the plasma level of the drug in infants and children treated for long periods of time.

KEY WORDS Anticonvulsants, developmental pharmacology, infant, phenobarbital, pre-school children.

Even if an exact correlation between plasma level and the therapeutic effect of phenobarbital (PB) is not established (1-5) the therapeutic concentration range is generally considered to be 10–25 µg/ml independent of the patient's age (1, 2, 3, 5).

A correlation was demonstrated between the plasma concentration and dosage of PB in adults. One mg/kg/day gives a plasma concentration of about 10 µg/ml, 2 mg/kg/day gives a concentration of about 20 µg/ml and so forth (1, 9). The PB dosage necessary to reach the same plasma level is higher in children than in adults and considering the relatively few data published, it seems that the younger the child, the greater the PB dosage needed to reach the same plasma level (10, 11). In fact, the plasma level/dosage ratio was found significantly higher at 10–14 years (6.9 ± 0.3 (S.D.)) than at 1–6 years (4.8 ± 0.2) (11).

There is however no exact knowledge about the correlation between the level/dosage ratio

of PB and the age of children during the first postnatal years. Some of our recent observations seem to be useful in clarifying the change in the ratio from 2 months up to 6 1/2 years of age.

METHODS

13 plasma PB concentrations were obtained from 82 children (2 months – 6 1/2 years old). All these patients were being treated for epilepsy or febrile convulsions and PB was administered once every 12 hours. No other drugs were administered. Twenty-six children had two or three drug determinations during a period from 3 to 8 months after the first determination. The greatest interval between the beginning of the therapy and the last drug administration was 7 years. All children were free of symptoms and signs of liver or kidney failure before and during therapy so that it can be assumed that major kidney or liver illnesses did not play a role in modifying the plasma level of PB (6, 8). In 31 children SGOT, SGPT, prothrombin activity, urea, creatinine, blood analysis and standard urine analysis could also be performed before the beginning of the therapy and were normal. Beginning after 3 weeks of therapy and with parental informed consent, blood for the determination of the drug level was drawn 5–7 hours after the last administration of the drug. The drug concentration was determined by gas-liquid chromatographic technique according to MacGee's method.

- 33 Penttilä I, Mäkelä H, A. Voutilinen A, J. Heikkinen E, Nummi S, Pystynen P & Siitonen J. Activities of aspartate and alanine aminotransferases and alkaline phosphatase in sera of healthy subjects. *Scand J Clin Lab Invest* 35: 275, 1975.
- 34 Schaller J & Wedgwood R J. Diseases of connective tissue (The so called collagen diseases). Juvenile rheumatoid arthritis. In S. S. Gellis & B. M. Kagan (eds). *Current pediatric therapy* 4. W. B. Saunders Company, Philadelphia 1970, p. 536.
- 35 Schaller J G & Wedgwood R J. Rheumatic diseases. Juvenile rheumatoid arthritis. In V. C. Vaughan & R. J. McKay (eds). *Nelson textbook of pediatrics*. W. B. Saunders Company, Philadelphia 1975, p. 530.
- 36 Shanks R. A. Collagen or connective tissue diseases. In J. O. Forfar & G. C. Arneil (eds). *Textbook of paediatrics*. Churchill Livingstone, Edinburgh 1973, p. 1676.
- 37 Sillanpää M, Mäkelä A. L. & Kouvikko A. Acute liver failure and encephalopathy (Reye's syndrome?) during salicylate therapy. *Acta Paediatr Scand* 64: 877, 1975.
- 38 Sills I. M. Diseases of connective tissue (The so called collagen diseases). In S. S. Gellis & B. M. Kagan (eds). *Current pediatric therapy* 7. W. B. Saunders Company, Philadelphia 1976, p. 360.
- 39 Stevens D. L. & Kaplan D. B. Salicylate intoxication in children: report of 4 cases. *Am J Dis Child* 70: 331, 1945.
- 40 Stollerman G. H. Connective tissue diseases. Juvenile rheumatoid arthritis. In H. I. Barnett & A. H. Einhorn (eds). *Pediatrics*. Appleton-Century-Crofts, New York 1972, 15 ed., p. 506.
- 41 Tikkanen R. & Strandman D. Cellulose acetate by phthalate CAP as enteric coating material. The absorption of acetylsalicylic acid from the CAP coated enteric tablets. *Farmaseuttinen Aikakauslehti Farmaseuttisk Notisblad* 74: 59, 1965.
- 42 Trinder P. Rapid determination of salicylate in biological fluids. *Biochem J* 57: 301, 1964.
- 43 Wedgwood R. J. & Schaller J. Juvenile rheumatoid arthritis (Still's disease). In W. E. Nelson & V. C. Vaughan & R. J. McKay (eds). *Textbook of pediatrics*. W. B. Saunders Company, Philadelphia 1969, p. 517.
- 44 Williams R. T. *Detoxication mechanisms*. Chapman & Hall, London 1959, 2nd ed., p. 359.
- 45 Wolfe J. D., Metzger A. L. & Goldstein R. L. Aspirin hepatitis. *Ann Intern Med* 80: 74, 1974.

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There is however no exact knowledge about the correlation between the level/dosage ratio

of PB and the age of children during the first postnatal years. Some of our recent observations seem to be useful in clarifying the change in the ratio from 2 months up to 6 1/2 years of age.

METHODS

13 plasma PB concentrations were obtained from 8 children (7 months – 6 1/2 years old). All these patients were being treated for epilepsy or febrile convulsions and PB was administered once every 12 hours. No other drugs were administered. Twenty-six children had two or three drug determinations during a period from 3 to 8 months after the first determination. The greatest interval between the beginning of the therapy and the last drug administration was 2 years. All children were free of symptoms and signs of liver or kidney failure before and during therapy so that it can be assumed that major kidney or liver illnesses did not play a role in modifying the plasma level of PB (6, 8). In 31 children SGOT, SGPT, prothrombin activity, urea, creatinine, blood analysis and standard urine analysis could also be performed before the beginning of the therapy and were normal. Beginning after 3 weeks of therapy and with parental informed consent, blood for the determination of the drug level was drawn 5–7 hours after the last administration of the drug. The drug concentration was determined by gas-liquid chromatographic technique according to MacGee's method.

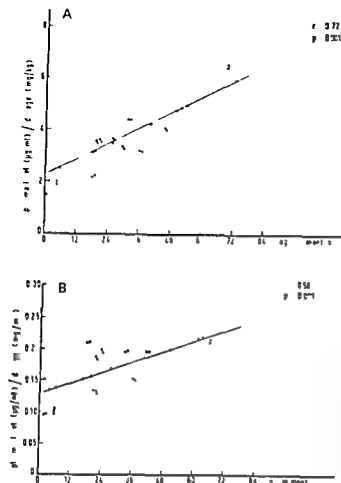


Fig. 1 Correlation between age and plasma level/dosage ratio for PB in children A dosage in mg/kg B dosage in mg/m²

od (7) A Frictionless Gas Chromatography Apparatus was employed equipped with a flame ionization detector (Carlo Erba). Exobarbital was used as an internal standard. PB was extracted with the following modified procedure: 1 ml of plasma acidified with 0.1 ml 1N HCl was extracted with 8 ml chloroform by gentle shaking for 30 min. 7 ml of clear chloroform layer added to 20 µg of internal standard (exobarbital 700 µg/ml ethanol solution) were dried under nitrogen stream in a water bath at 40 °C. The residue was dissolved in 50 µl of tetramethyl ammonium and 1-2 µl were injected into the gas chromatograph. A calibration curve (5-30 µg/ml in fresh plasma) was carried out each time with unknown samples.

Table 1 Plasma level/dosage ratios of PB for three different age groups

The dosage of PB is calculated in mg/kg

2 months-1 year	1-3 years	3-6 1/2 years
2.31 ± 0.74 (S.D.) (S.E. 95% = 0.31)	3.54 ± 0.99 (S.D.) (S.E. 95% = 0.24)	4.79 ± 1.31 (S.D.) (S.E. 95% = 0.40)

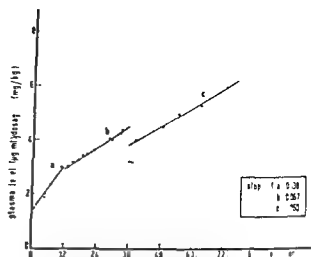


Fig. 2 Correlations between age and plasma level/dosage ratio for PB for surface area related dosages in the 3 groups of children divided according to their age

The correlations of the plasma level/dosage ratio with the age of the patient were calculated both for weight and surface area related dosages. The ratios for weight related dosages within the three groups of children divided according to age (2 months-1 year, 1-3 years, 3-6 1/2 years) were also calculated as were their correlations with the age of the children in each group. The dosage of PB in mg/kg/day required at the different ages in order to have plasma level of 10-25 µg/ml respectively was calculated from these ratios.

RESULTS

Fig. 1 shows the correlations of the plasma level/dosage ratio with the age of the patient both for weight and surface area related dosages. The two correlations are highly significant ($p < 0.001$). The plasma level/dosage ratio increases as the age of the patient increases, this increase being greater for dosage in mg/kg than for dosage in mg/m². Table 1 shows the plasma level/dosage ratios for weight related dosages in the 3 groups of children divided according to age (2 months-1 year, 1-3 years, 3-6 1/2 years). These ratios maintain a significant correlation with the age of the patient ($p < 0.01$ for the 3 lines, this significance being lower than that of the entire group as each individual group has fewer data). Fig. 2 shows the 3 lines with their respective slopes. It is clear that the rate of increase of plasma level/dosage ratio shown to

Table 2 Dosages of PB in mg/kg/day required at the different ages in order to have plasma levels of 10–25 µg/ml respectively

months–1 year	1–3 years	3–6½ years
4–11 mg/kg	3–7 mg/kg	2–5 mg/kg

exist with the increase in age diminishes after the first year

The plasma level/dosage ratios for surface area related dosages have no significant correlation with the ages of the children in the 3 groups because the data were too dispersed

DISCUSSION

Our data prove that in steady state conditions and in the absence of liver and kidney illnesses from 2 months up to 6 1/2 years of age the plasma level/dosage ratio for PB has a statistically significant increase with the increase of the patient's age both for dosage in mg/kg and in mg/m². Moreover at least for body weight related dosages this increase is relatively high in the first year of life becoming less marked thereafter

From a practical point of view the dosages in mg/kg/day required to have PB level of 10–25 µg/ml in the 3 groups of children divided according to their age (from 2 months up to 6 1/2 years) are shown in Table 2

The increase of plasma level/dosage ratio for PB as the patient gets older presumably depends on the variations of drug metabolism during development. The apparent plasma half life of the drug increases with the increase in age (4 9 10 11) and this seems to be the most important factor. The role of volume of distribution variation is still undetermined as it was never thoroughly evaluated in the age period we have considered

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dren should have been hospitalized for at least 3 weeks before each drug determination. This was an impossible procedure for practical and ethical reasons. Therefore the variability of our data may be in part due to the irregular administration of the drug in some children. On the other hand our data are so significant that it seems highly improbable to us that a lack of compliance plays an important role. Considering the variability among individuals and the speed with which the level/dosage ratio for PB varies during growth it is recommended to regularly measure the plasma level of the drug in infants and children treated for long periods of time. Only in this way can an adequate dosage be maintained

ACKNOWLEDGEMENT

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REFERENCES

- Buchthal F & Lennox Buchthal M A. Phenobarbital. Relation of serum concentration to control of seizures. In D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs*. Raven Press New York 1973 p 335
- Buchthal F, Svensmark P & Simonsen M. Relation of EEG and seizures to phenobarbital in serum. *Arch Neurol* 19 567 1968
- Faero O, Kastrup K W, Melchior J C & Thorm J. Phenobarbital as prophylaxis for febrile convulsions. *Epilepsia* 12 109 1971
- Garrettson L K & Dayton P G. Disappearance of phenobarbital and diphenylhydantoin from serum in children. *Clin Pharmacol Ther* 2 674 1970
- Jalling B. Plasma concentrations of phenobarbital in the treatment of seizures in newborns. *Acta Paediatr Scand* 64 514 1975
- Kott H, Winters W S, Herman H & McDowell F. Diphenylhydantoin and phenobarbital toxicity. *Arch Neurol* 11 649 1964
- MacGee J. Rapid determination of diphenylhydantoin in blood plasma by gas liquid chromatography. *Anal Chem* 42 4 1 1970
- Mayneri E W. Phenobarbital, mephobarbital and methobarbital. Absorption, distribution and excretion. In D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs*. Raven Press New York 1973 p 303

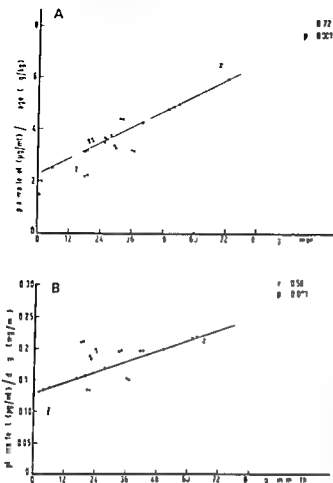


Fig. 1 Correlation between age and plasma level/dosage ratio for PB in children. A dosage in mg/kg B dosage in mg/m²

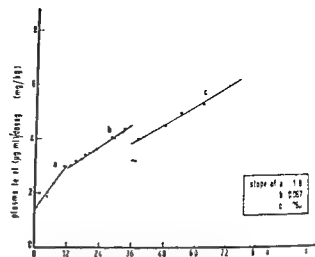


Fig. 2 Correlations between age and plasma level/dosage ratios for PB for weight related dosages in the 3 groups of children divided according to their age

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Table 7 Dosages of PB in mg/kg/day required at the different ages in order to have plasma levels of 10–25 µg/ml respectively

1 month–1 year	1–3 years	3–6½ years
4–11 mg/kg	3–7 mg/kg	2–5 mg/kg

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The plasma level/dosage ratios for surface area related dosages have no significant correlation with the ages of the children in the 3 groups because the data were too dispersed.

DISCUSSION

Our data prove that in steady state conditions and in the absence of liver and kidney illnesses from 2 months up to 6½ years of age the plasma level/dosage ratio for PB has a statistically significant increase with the increase of the patient's age both for dosage in mg/kg and in mg/m². Moreover at least for body weight related dosages this increase is relatively high in the first year of life becoming less marked thereafter.

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REFERENCES

- 1 Buchthal F & Lennox Buchthal M A Phenobarbital: Relation of serum concentration to control of seizures. In D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs*. Raven Press, New York 1977, p 315.
- 2 Buchthal F, Svensmark P & Simonen H Relation of EEG and seizures to phenobarbital in serum. *Arch Neurol* 19: 567, 1968.
- 3 Faero O, Kasirup K W, Melchior J C & Thorm J Phenobarbital as prophylaxis for febrile convulsions. *Epilepsia* 17: 109, 1971.
- 4 Garretson I K & Dayton P G Disappearance of phenobarbital and diphenylhydantoin from serum of children. *Clin Pharmacol Ther* 2: 674, 1970.
- 5 Jalling B Plasma concentrations of phenobarbital in the treatment of seizures in newborns. *Acta Paediatr Scand* 64: 514, 1975.
- 6 Kutt H, Winters W, Scherman R & McDowell F Diphenylhydantoin and phenobarbital toxicity. *Arch Neurol* 11: 649, 1964.
- 7 MacGee J Rapid determination of diphenylhydantoin in blood plasma by gas liquid chromatography. *Anal Chem* 47: 421, 1970.
- 8 Maynert E W Phenobarbital, mephobarbital and methobarbital: Absorption, distribution and excretion. In D M Woodbury J K Penry & R P Schmidt (eds) *Antiepileptic drugs*. Raven Press, New York 1977, p 303.

- 9 Svensmark O & Buchthal F Accumulation of phenobarbital in man *Epilepsia* 4 199 1963
 - 10 Svensmark O & Buchthal F Dosage of phenytoin and phenobarbital in children *Dan Med Bull* 10 234 1963
 - 11 Svensmark O & Buchthal F Diphenylhydantoin and phenobarbital Serum levels in children *Am J Dis Child* 108 82 1964
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SERUM CONCENTRATIONS OF THEOPHYLLINE IN CHILDREN FOLLOWING THE ADMINISTRATION OF DOSES GENERALLY RECOMMENDED NEW DOSAGE REGIMEN REQUIRED

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ABSTRACT Selvig K, Lingaas Holmen T, Aas K, Rugstad H E and Bierve K (Institute of Clinical Biochemistry, the Allergy Institute, Voksentoppen, Department of Paediatrics and Department of Surgical Research, Section of Clinical Pharmacology, Rikshospitalet, National Hospital of Norway, Oslo, Norway). Serum concentrations of theophylline in children following the administration of doses generally recommended: new dosage regimen required. *Acta Paediatr Scand* 68: 435-1979. —Serum concentrations of theophylline following intravenous and oral administration of aminophylline were studied in asthmatic children 2-17 years of age. The biological half life ($t_{1/2}$) of theophylline varied between 165 and 490 min. The results revealed that an intravenous loading dose of 6 mg of aminophylline per kg body weight was necessary in order to obtain therapeutic concentrations in children who had not received the drug for the last 6-8 hours. The maintenance dose should be determined and controlled by use of serum concentration determinations. In a group of children receiving 5 mg of aminophylline per kg body weight 3 times a day orally, none had concentrations within the therapeutic range in the morning, and only 39% reached therapeutic levels 2 h after the morning dose. No correlation was found between the serum concentration of theophylline and the amount of drug given per kg body weight. The results show that theophylline concentration analysis is necessary to obtain adequate therapeutic levels in children without risking toxic effects.

KEY WORDS Theophylline, children, asthma, serum drug concentration

Theophylline is known to be an effective bronchodilator and is widely used in the treatment of bronchospasm in asthma and chronic obstructive lung disease. The drug is often administered as aminophylline, the ethylene diamine salt of theophylline.

The effect of theophylline on pulmonary function is related to the serum concentration, and 55 to 110 $\mu\text{mol/l}$ has been accepted as the therapeutic range (6-9, 13). The therapeutic index of theophylline is narrow, and the frequency of gastrointestinal, cerebral, nervous and cardiovascular side effects is high at concentrations above the therapeutic range. Serious and occasionally fatal effects such as convulsions, atrial and ventricular arrhythmias and hypo- or hypertension have been re-

ported at concentrations above 135 $\mu\text{mol/l}$ (4, 16).

Theophylline is biotransformed in the liver, and the more water soluble metabolites are excreted in the urine (1, 5). Only 10% of the drug is excreted unchanged in the urine. There is a large interindividual variation in the rate of theophylline elimination (6, 10), which is mainly due to different rates of metabolism. In children, the mean biologic half life is shorter than in adults (2, 15), and therefore they usually need shorter dose intervals on higher doses than adults to maintain therapeutic serum concentrations.

We have studied the drug concentrations in serum obtained after administration of conventional doses of theophylline to children.

- 9 Svensmark O & Buchthal F Accumulation of phenobarbital in man *Epilepsia* 4: 199-1963
 - 10 Svensmark O & Buchthal F Dosage of phenytoin and phenobarbital in children *Dan Med Bull* 10: 234-1963
 - 11 Svensmark O & Buchthal F Diphenylhydantoin and phenobarbital Serum levels in children *Am J Dis Child* 108: 82-1964
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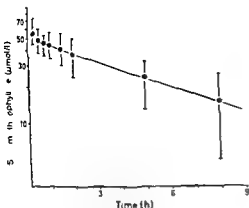


Fig. 1 Serum theophylline concentration as a function of time after intravenous injection of 5 mg of aminophylline per kg body weight. Mean and range from 16 children are given.

line per kg body weight ranged from 45 to 73 $\mu\text{mol/l}$ and 4 to 23 $\mu\text{mol/l}$ respectively. The apparent volume of distribution varied from 0.37 to 0.53 l/kg while the biologic half life (β phase) varied from 165 to 495 min (similar to previously reported results (2, 15)). Table 2 shows that the individual biologic half life (β phase) of theophylline in children was independent of dose over the range from 3 mg to 5 mg of aminophylline per kg body weight i.e. there was no discernable dose dependent kinetics in this dose range and the increase in serum concentration was proportional to the increase in drug dose. The mean biologic half life of theophylline in children receiving no other drugs was not statistically

different from the half life found in children receiving simultaneous administration of therapeutic doses of salbutamol, adrenaline, corticosteroids or disodium cromoglycate (291 min and 294 min respectively).

The results following oral treatment with aminophylline are shown in Fig. 2. The mean daily dose of aminophylline was 16.3 mg/kg body weight ranging from 10.3 to 23.2 mg/kg. This corresponds to 12.8 (8.1–18.3) mg of theophylline per kg body weight. No correlation could be found between such total daily doses of theophylline and the corresponding serum concentrations neither before the morning dose nor at the expected peak concentration 2 hours later. The serum concentration was also measured 4 and 8 hours after the morning dose and again no correlation with the daily dose was found (results not shown).

The serum concentration of theophylline fluctuated considerably during the day and this was strongly influenced by the dose intervals used. The children who had the drug administered 3 times during a period of 11 hours had very low serum concentrations in the morning and accumulated the drug during the day reaching a maximum serum concentration after the evening dose in contrast to children receiving the drug every 8 hours who showed a less pronounced fluctuation of the serum concentration. However although those who received the last dose at 1 a.m.

Table 2 Changes in the serum concentration and biologic half life after increase in dose of theophylline

4 children received an intravenous dose of 3 mg and after an interval of at least 4 days 5 mg of aminophylline per kg body weight (corresponding to 4 mg and 3.9 mg theophylline respectively) a dose increase of 63%.

Patient no.	Theophylline concentration (\sim increase)			Biologic half life: min (β phase)	
	15 min	90 min	5 h	3 mg/kg	5 mg/kg
1	59	56	79	31	316
2	36	41	67	180	80
3	70	65	57	64	58
4	9	70	75	6	86

Table 1 Serum concentrations of theophylline obtained in children 15 min after start of a single intravenous dose of aminophylline

Dose of aminophylline (mg/kg body weight)	No. of children	Theophylline concentration after 15 min ($\mu\text{mol/l}$) ^a	Time within therapeutic area (min)
3 (2-4)	4	33 (33-34)	<15
5 (3-9)	16	55 (37-73)	20 (<15-75)
6 (4-7)	5	61 (47-72)	65 (<15-170)

^a Dose recalculated as theophylline

^b Mean and range are given

^c Time within therapeutic area is calculated from semilogarithmic plots of serum concentrations versus time. Median and range are given

SUBJECTS AND METHODS

This study includes 68 children, age 2-17 years, all hospitalized with a diagnosis of bronchial asthma. Both children and parents had consented to the study.

Intravenous administration Twenty-one children, age 8-15 years, without any other known illness were included in this study. They did not receive xanthine containing drugs for 72 h prior to the study. Eleven of the children received other bronchodilators, corticosteroids or cromoglycate, but these drugs were discontinued 6 h before the study. Aminophylline, 3, 5 or 6 mg per kg body weight, was injected over a period of 10 min. Four of the children received both 3 mg and, after an interval of at least 4 days, 5 mg per kg body weight. A Venflon[®] was installed in the opposite arm, and 4 ml blood were sampled before and then 15, 30, 45, 60, 90, 2 h, 5 h, 8 h and in some cases 12 h and 24 h after the start of the injection. The lung function, measured as FEV₁ and the pulse rate were registered during the test period.

Oral administration Serum concentrations of theophylline were studied in a non-selected group of 47 children admitted to the hospital and treated with aminophylline. The mean daily dose was 16.3 mg/kg body weight (range 10.3-23.2). The drug was administered 2 or 3 times a day for at least 72 h before the test. Blood samples were taken immediately before and 2 h after the morning dose. Some of the children used bronchodilators, corticosteroids or cromoglycate. No change was made in this therapy during the study.

Analytical method The serum concentration of theophylline was determined by cation exchange HPLC as previously described (12).

RESULTS

The serum concentrations of theophylline after intravenous injection of 3, 5 or 6 mg of aminophylline per kg body weight are shown in Table 1. Because of the very slow intrave-

nous injection of the drug, it is assumed that the serum concentration 15 min after start of the injection gives a good estimate of the maximum concentration reached in each child. To obtain therapeutic concentrations of theophylline, it was necessary to give at least 5 mg of aminophylline per kg body weight as a single loading dose. At this dose, 62% of the children reached assumed therapeutic values 15 min after the start of the injection. Only in 1 patient did the concentration remain within the therapeutic range for more than 1 hour. In all children, including 5 receiving 6 mg/kg body weight, the maximum serum concentration obtained was in the lower half of the therapeutic range.

Table 1 illustrates the large variations in serum concentrations of theophylline following administration at three dose levels. This is further shown in Fig. 1 in which a semilogarithmic plot of the mean serum concentration after a loading dose of 5 mg of aminophylline per kg body weight in 16 children is given. After a rapid α phase (mean $t_{1/2}$ 10 min), the elimination curve was rectilinear, but the slope of the β phase varied as demonstrated by the increasing range of the serum theophylline values with time. Thus, the theophylline concentration 15 min and 8 hours after a single injection of 5 mg of aminophylline

Aminophylline AFI (30 mg/ml)

^a Theodrox 3M Riker (aminophyllin 195 mg/tbl)

act this by means of therapeutic serum concentrations also in the morning aminophylline must be administered 3 or 4 times during 24 hours. However in most children it is not possible to give any medication during the night which necessarily results in subtherapeutic concentrations in the morning. This problem could hopefully be overcome using a sustained release preparation.

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REFERENCES

- 1 Cornish H H & Christman A A. A study of the metabolism of theobromine, theophylline and caffeine in man. *J Biol Chem* 228 315 1957.
- 2 Ellis E F, Koysooko R & Levy G. Pharmacokinetics of theophylline in children with asthma. *Pediatrics* 58 547 1976.
- 3 Fixley M, Shen D D & Azarnoff D L. Theophylline bioavailability. A comparison of the oral absorption of a theophylline elixir and two combination theophylline tablets to intravenous aminophylline. *Am Rev Resp Dis* 115 955 1977.
- 4 Jacobs M H, Senior R M & Kesler G. Clinical experience with theophylline. Relationship between dosage, serum concentration and toxicity. *JAMA* 235 1983 1976.
- 5 Jenne J W, Nagasawa H T & Thompson R H. Relationship of urinary metabolites of theophylline to serum theophylline levels. *Clin Pharmacol Ther* 19 374 1976.
- 6 Jenne J W, Wyse E, Rood F S & MacDonald F M. Pharmacokinetics of theophylline. Application to adjustment of the clinical dose of aminophylline. *Clin Pharmacol Ther* 13 349 1977.
- 7 Kern J W & Lipman A G. Rational theophylline therapy. *Drug Intell Clin Pharm* 11 144 1977.
- 8 Kordash T H, Van Dellen R G & McCall J T. Theophylline concentrations in asthmatic patients after administration of aminophylline. *JAMA* 239 139 1977.
- 9 Mitenko P A & Ogilvie R I. Rational intravenous doses of theophylline. *N Engl J Med* 289 600 1973.
- 10 Mitenko P A & Ogilvie R I. Pharmacokinetics of intravenous theophylline. *Clin Pharmacol Ther* 14 509 1973.
- 11 Pfafsky K M & Ogilvie R I. Dosage of theophylline in bronchial asthma. *N Engl J Med* 297 1718 1975.
- 12 Selvig K & Byrve K E. Determination of serum propylxanthines by high pressure liquid chromatography. *Scand J Lab Invest* 37 373 1977.
- 13 Weinberger M M & Bronsky E A. Evaluation of oral bronchodilator therapy in asthmatic children. *J Pediatr* 84 421 1974.
- 14 Welling P G, Lyons L L, Craig W A & Trochta G A. Influence of diet and fluid on bioavailability of theophylline. *Clin Pharmacol Ther* 17 475 1975.
- 15 Zaske D E, Miller K W, Strem L L, Austrian S & Johnson P H. Oral aminophylline therapy. Increased dosage requirements in children. *JAMA* 237 1453 1977.
- 16 Zwillich C W, Sutton F D, Neff T A, Cohn W M, Matthay R A & Weinberger M M. Theophylline induced seizures in adults. Correlation with serum concentrations. *Ann Intern Med* 87 784 1975.

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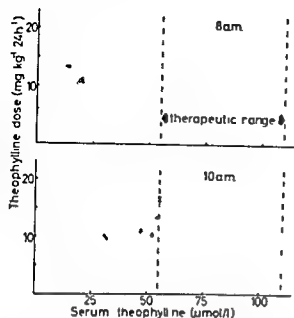


Fig. 2 Relation between the daily dose of theophylline and the serum concentration in 47 children after oral administration. The drug was administered 2 or 3 times daily with the first dose at 7 p.m. (O) or 3 times daily with the first dose at 1 a.m. (●). Serum concentrations were determined immediately before the morning dose (8 a.m.) and 2 h afterwards (10 a.m.).

tended to have a higher concentration all children had subtherapeutic serum concentrations in the morning as shown in Fig. 2.

None of our patients had serum theophylline concentrations above the therapeutic range and no side effects were registered in the ward. One child complained of gastrointestinal pain after he had been discharged from the hospital. On control he had a serum concentration of 47 µmol/l.

DISCUSSION

The recommended intravenous dose of aminophylline in the treatment of bronchial asthma has been 3 mg/kg body weight corresponding to 2.4 mg of theophylline. This dose has mainly been selected from clinical data excluding the higher doses following reports of side effects. Our results show that the recommended dose does not produce therapeutic serum concentrations. In children who have not previously received theophylline therapy a loading dose of 6 mg of aminophylline per

kg body weight seems to be more adequate confirming results reported by other groups (9, 11). In patients already on oral or rectal aminophylline treatment the loading dose should usually be somewhat lower and in some children even omitted to avoid toxic serum concentrations.

Maintenance therapy can be given either as repeated intravenous injections or as continuous intravenous infusions. The infusion dose proposed to provide therapeutic serum theophylline concentrations is 0.9 mg/kg/h in adults (9). However, this dose gives theophylline concentrations above the upper therapeutic limit in more than 30% of the patients (8). Children tend to require somewhat higher doses because of a higher total clearance of the drug (2). However, because of the pronounced interindividual differences in the kinetics of the drug (Fig. 1) it is necessary to adjust the individual dose based on serum concentration determinations.

The same interindividual differences are found after oral administration of aminophylline. In this study no correlation was found between the total daily dose and the serum concentration, but some of this discrepancy may also be due to different rates of absorption of the drug (3). In other studies on the bioavailability of theophylline the peak serum concentration was reached 1 to 2 hours after oral administration (3, 7). This can however be strongly influenced by the diet and intake of fluid (14). In our group of children 30% were still in the absorption phase 2 hours after oral administration of the drug as indicated by a higher concentration 4 hours after administration.

The routinely used dose of aminophylline has been 5 mg/kg body weight 3 times daily. On this dose none of the children we studied had a serum concentration within the therapeutic area in the morning and only 39% had reached therapeutic levels 2 hours after the morning dose. Bronchial obstruction on awakening in the morning is a serious problem in children with chronic asthma. To counter

SHORT COMMUNICATION

SALT CONTENT IN HUMAN BREAST MILK DURING THE THREE FIRST WEEKS AFTER DELIVERY

During a study of electrolyte balance in new born infants the concentration of sodium and potassium in breast milk was determined on different days after delivery

Material and Methods Mothers who were delivered at term were studied on the first ($n=5$) second ($n=5$) third ($n=17$) fourth ($n=14$) fifth ($n=19$) sixth ($n=6$) seventh ($n=5$) eighth and ninth ($n=5$) tenth and eleventh ($n=5$) and twenty second to twenty sixth ($n=12$) days after delivery. In addition 19 samples were obtained 1-11 days after delivery from mothers who were delivered during the 32nd-34th gestational week. A 1 ml sample of breast milk was obtained at the beginning and/or at the end of the feeding. The concentration of sodium and potassium in 1/50 diluted breast milk was determined with a flame photometer

Results and Comments In 11 mothers delivered at term and studied 3-6 days after delivery the concentration of sodium and potas-

sium in breast milk was determined both before and after feeding. Paired t tests showed no significant differences between the samples ($\text{Na } p>0.9$ $\text{K } 0.5>p>0.4$). The concentration of sodium in breast milk (Colostrum) of mothers who were delivered at term was high 61 mmol/l as expected on the first day of life (6). It then fell to about 20 mmol/l at the second or third day of life and remained at this level until at least the 11th day after delivery (Fig. 1). The average sodium concentration in breast milk from the 10th to the 11th day after delivery 24.6 mmol/l was significantly higher than the average sodium concentration in breast milk 3-4 days after delivery 5.1 mmol/l ($p<0.001$). The sodium concentration in breast milk 3-4 weeks after delivery was in the same range as that reported previously (3, 6). The sodium concentration of breast milk in mothers delivered before term was also comparatively higher during the first weeks after delivery but was somewhat lower than that of breast milk from mothers who were delivered at term (Fig. 2). The potassium concentration of breast milk is fairly constant after delivery. The potassium sodium ratio in breast milk is lower during the first 10 days after delivery than later during lactation (Fig. 3).

The results also suggest that infants who are not breast fed should be given salt supplements. This is probably more important for preterm infants since they have greater urinary salt losses than the full term infants (1, 2, 8).

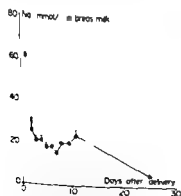


Fig. 1. Sodium content in breast milk from mothers who are delivered at term. The dots represent mean values and the bars \pm SE.

Supported by grants from the Swedish Medical Research Council (B78-19X-01644-06) and from the Research Funds of the Karolinska Institute.

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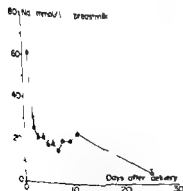


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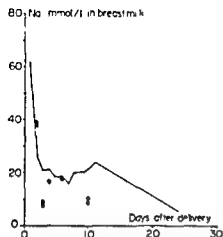


Fig. 3 Sodium content in breast milk from mothers that are delivered during the 32nd-34th gestational week (6). The line represents the average values observed in breast milk from mothers that are delivered at term.

and easily develop hyponatremia (5-7). A sodium supplement has been reported to induce accelerated growth in pre-term infants (4). The findings reported indicate that an increased active reabsorption of sodium in the mammary gland is responsible for the fall in sodium concentration of breast milk after the 10th day of delivery. The parallel changes in the potassium sodium ratio indicate that this is accomplished by an increased sodium potassium exchange.

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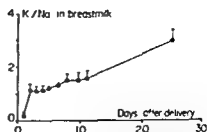


Fig. 4 Potassium sodium ratio in breast milk from mothers that are delivered at term. The dots represent the mean value and the bar 1 S.E.

REFERENCES

- 1 Aperia A, Broberger O, Herin P & Zetterstrom R. Salt balance and aldosterone excretion during the first two weeks of life in preterm and full term infants. *Acta Paediatr Scand* submitted for publication.
- 2 Arant B S Jr. Developmental patterns of renal functional maturation compared in the human neonate. *J Pediatr* 92: 705, 1978.
- 3 Bibson G. Feeding the low birth weight infant. *J Pediatr* 79: 694, 1971.
- 4 Chance G W, Radde I C, Willis D M & Park E. Postnatal growth of infants of <1.3 kg birth weight. Effects of metabolic acidosis, of caloric intake and of calcium, sodium and phosphate supplementation. *J Pediatr* 91: 747, 1977.
- 5 Day G M, Radde I C, Balfe J W & Chance G W. Electrolyte abnormalities in very low birth weight infants. *Pediatr Res* 10: 525, 1976.
- 6 Renner E. *Milch und Milchprodukte in der Ernährung des Menschen*. Volkswirtschaftlicher Verlag GmbH Krampton Verlag, Th. Munn OHG, Hildesheim, 1974.
- 7 Roy R N, Chance G W, Radde I C, Hill D E, Willis D M & Shepers J. Late hyponatremia in very low birthweight infants (<1.3 kg). *Pediatr Res* 10: 556, 1976.
- 8 Ross B, Cowett R M & Oh W. Renal functions in low birth weight infants during the first two months of life. *Pediatr Res* 11: 1165, 1977.

SHORT COMMUNICATION

POLYCYSTIC KIDNEY DISEASE OF PERINATAL TYPE

Polycystic kidney disease of childhood is a recessively inherited heterogenous entity associated with a specific hepatic lesion (2-7). The most familiar and accepted form is the perinatal type of polycystic kidney disease of childhood (PPKD). Larroche calculated a PPKD frequency of $1/3/10^3$ child autopsies (7). We describe nine cases of PPKD in our child autopsy material.

The study was based on hospital and autopsy records of five girls and four boys from seven different families with PPKD diagnosed and treated in our hospital during the years 1965-1977. We encountered no other types of polycystic kidney disease of childhood in our child autopsy material. The mothers had a total of 29 pregnancies and 22 children including these newborns. Of these 22 children 13 had clinically obvious PPKD but four died in other hospitals or before the year 1965 and were thus excluded from the study.

During the time of the survey 1524 child autopsies were performed. Thus the frequency of PPKD was $5.9/10^3$ autopsies. The incidence was $13.3/10^3$ births.

The gestational age ranged from 33 weeks to 40 weeks. Four of these newborns were de-

livered by breech presentation. Birth weight ranged from 1900 to 4140 g (Table 1). Oligohydramnios was found in all but one of the cases in which the amount of amniotic fluid was recorded. The placenta was large in seven cases, the mean placental/fetal ratio being 31% and the range from 20 to 47% (Table 1). Survival time ranged from one to 34 hours and only one child lived over 24 hours.

The lungs were atelectatic with haemorrhages. Mild lung hypoplasia was found in two cases. Other congenital malformations included talipes equinovarus in two cases, mild hydrocephalus, atrial septal defect and hypoplastic adrenal glands with a total weight of 2 g.

Histologically the typical liver and renal findings were observed in all cases. In six cases the pancreas specimen was available. It showed dense periductal and lobular fibrosis, the degree of fibrosis varying little from case to case (Table 1). The placenta was histologically immature. The histological picture was similar to that in congenital nephrosis of the Finnish type (6).

The incidence of PPKD seems to be high in our material and is similar to the incidence of

Table 1. Clinical and pathological findings of children with PPKD

Case	Family	Sex	Weight (g)	Weight of kidneys (g)	Fibrosis of pancreas	Placental/fetal ratio (%)	Abnormal placental histology
I	I	F	1900	93	+	47	
2	I	F	040	136	+	47	Not known
3	II	M	3370	343	+	47	+
4	II	M	4140	64	Not known	21	+
5	III	F	3190	400	+	0	+
6	IV	F	3050	150	Not known	36	+
7	V	F	760	190	+	34	+
8	VI	M	600	255	Not known	27	Not known
9	VII	F	3140	63	+	25	Not known
					+	27	+
							Not known

congenital nephrosis of the Finnish type in Finland (4). We found large placenta in our series and the same finding has been reported in the congenital nephrosis too. Thus it seems that congenital renal diseases can cause large placenta. Furthermore the breech presentation can be caused by big renal masses and the large placenta which blocks the turning of the fetus to the vertex position.

As in earlier studies the segregation was compatible with autosomal recessive inheritance (2, 8). Therefore in genetic counseling of PPKD it is important to tell parents that only future sibs may be affected. In these families fetal sonography is indicated since the polycystic kidneys of the fetus sometimes can be recognized early enough to allow the parents to elect therapeutic abortion (1, 3). It has also been reported that the dysplastic kidneys excrete excessive amounts of alpha₁-fetoprotein which can be found in amniotic fluid (5).

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REFERENCES

- 1 Bartley J A, Golbus M B, Filley R A & Hall J D. Prenatal diagnosis of dysplastic kidney disease. *Clin Genet* 11: 375, 1977.
- 2 Blyth H & Ockenden B G. Polycystic disease of kidneys and liver presenting in childhood. *J Med Genet* 8: 257, 1971.
- 3 Garret W J, Grunwald H & Robinson D E. Prenatal diagnosis of fetal polycystic kidney by ultrasound. *Aust N Z J Obstet Gynaecol* 10: 1, 1970.
- 4 Huttunen N P. Congenital nephrotic syndrome of Finnish type. Study of 75 patients. *Arch Dis Child* 51: 344, 1976.
- 5 Kasse S, Rose J S, Godmilow L, Walker B A, Kerzner T, Beratis N, Reyes P & Hirschhorn K. Prenatal diagnosis of renal anomalies. *Am J Med Genet* 1: 241, 1977.
- 6 Kouvvalainen K, Hjelt L & Hallman N. Placenta in congenital nephrotic syndrome. *Ann Paediatr Fenn* 8: 161, 1967.
- 7 Larroche J C. Developmental pathology of the neonate. Excerpta Medica, Amsterdam 1977, p. 96.
- 8 Lundin P M & Olow I. Polycystic kidneys in newborn infants and children. A clinical and pathological study. *Acta Paediatr Scand* 50: 185, 1961.

CASE REPORT

PARTIAL TRISOMY 15 AND INTRACTABLE SEIZURES

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ABSTRACT Taysi K Devivo D C and Sekhon G ■ (The Edward Mallinckrodt Department of Pediatrics and the Department of Neurology and Neurosurgery (Neurology) Washington University School of Medicine and the Divisions of Medical Genetics and Neurology St Louis Children's Hospital St Louis Missouri USA) Partial trisomy 15 and intractable seizures *Acta Paediatr Scand* 68 445 1979 —The clinical and cytogenetic findings of a case with partial trisomy of chromosome 15 are presented. Dysmorphic features of this newly recognized syndrome are too nonspecific and mild to permit the physician to make clinical diagnosis or to consider obtaining cytogenetic studies in early life. However, the present case indicates that seizures represent an important part of the clinical presentation of this rare chromosomal syndrome in addition to severe mental retardation.

KEY WORDS Partial trisomy chromosome 15 seizures

Twenty-one cases of partial trisomy of chromosome No. 15 have been reported in the cytogenetic literature since the initial description by Webb and colleagues in 1971 (7). The clinical features of this abnormality are not sufficiently distinctive to permit the physician to make the diagnosis clinically or to encourage one to obtain cytogenetic studies. Consequently, the majority of patients have not been diagnosed until they reach school age. We uncovered another case of partial trisomy involving the long arm of chromosome No. 15 while evaluating a young girl with intractable seizures, profound psychomotor retardation and dysmorphic features. The purpose of this report is to call attention to the fact that seizures represented an important part of the clinical picture in our patient and in approximately 50% of the reported cases (1-6, 8).

CASE REPORT

The patient, an 8 year-old black female, was born to an 11 year-old primigravida. There was no known exposure to drugs, alcohol or other known teratogens during the

uncomplicated pregnancy. The infant was born at term by vaginal delivery in breech presentation after an uncomplicated labor. The birth weight was 3 60 g and the neonatal period was normal. However, she was irritable and fed poorly. She suffered a grand mal seizure at 7 months of age. Since then, she has had recurrent grand mal seizures with variable frequency, 1-2 per day to none for several months.

Her development has been delayed. She sat without support at 1 year, walked clumsily at 1½ years, was toilet trained at 2½ years and started to use simple sentences at 3 years of age. There has been no remarkable developmental progress since then. She has been evaluated extensively for the seizures which became progressively resistant to various medications including phenobarbital, phenytoin, carbamazepine and clonazepam in various doses and combinations. She was admitted to St. Louis Children's Hospital for further evaluation and treatment with a ketogenic diet. While on the ketogenic diet for one year, the seizure frequency decreased approximately 90%. Recent addition of dipropylacetic acid to the treatment regimen has effected further control of the seizures.

The family history is negative for birth defects, mental retardation and seizures. Her two younger siblings and both parents are healthy. There is no consanguinity.

The physical examination revealed a hyperactive, mentally retarded girl with an ataxic gait. Her height/weight

Abbreviations GTG=G banding of chromosomes by trypsin using Giemsa; QFQ=Q banding of chromosomes by fluorescence using quinacrine; RHA=R banding of chromosomes by heating using acridine orange.



Fig 1 Patient at 8 years of age

and head circumference were below the third percentile for age. Certain dysmorphic features were noted including frontal bossing, decreased bifrontal diameter, flat occiput, prominent supraorbital ridges, heavy eyebrows, synophrys, telecanthus (inner canthal distance 3.4 cm), slight antimongoloid slanting of the palpebral fissures, bilateral epicanthic folds, slightly low set and posteriorly rotated ears with prominent antihelix and high arched palate (Fig. 1). There was bilateral clinodactyly of the 5th fingers and mild syndactyly between the second and third toes bilaterally. A small umbilical hernia also was noted. The dermatoglyphic analysis was unremarkable. Mental and motor retardation and poor motor coordination characterized the neurological findings.

Psychometric evaluation revealed the following results: DQ 20 (Cuttel), SQ 39 (Vineland) and IQ 48 (Verbal Language). Normal laboratory studies included a com-

plete blood count, electrolytes, BUN, glucose, Ca, Ph, Mg, uric acid, creatinine, alkaline phosphatase, serum proteins, urine amino acids, and thyroid function tests. A skull X-ray, cerebral radionuclide angiogram and computed tomography were normal and the bone age was commensurate with the chronological age. The severely abnormal EEG showed multifocal independent spikes and nonfocal bursts of spike and slow waves superimposed upon a slow background.

CYTOGENETIC STUDIES

A chromosome analysis was performed on peripheral blood cultures of the patient and both parents. GTG, QFQ and RHA banding

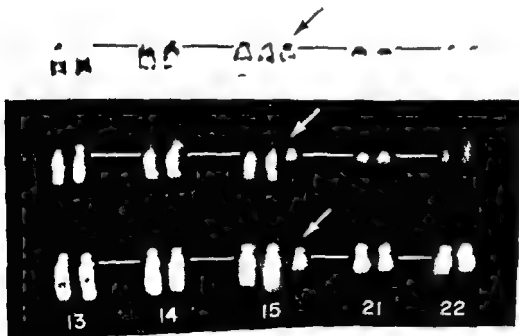


Fig 2 In descending order, GTG, QFQ and RHA banded partial karyotypes of the patient with extra No. 15 chromosome. The break occurred in band 2 of region 2 of the long arm of the extra chromosome 15.

techniques were employed in evaluating the patient's chromosomes

A small extra acrocentric chromosome was found in each of the patient's cells examined. Partial GTG QFQ and RHA banded karyotypes are shown in Fig 2. Comparing different karyotypic banding patterns allowed us to identify the extra chromosome as No. 15 with a deletion from band 22 of the long arm. The patient was therefore trisomic for the pter→q22 segment of chromosome No. 15: 47,XX+15del(15)(q22).

Chromosome studies from both parents yielded normal karyotypes with GTG banding

DISCUSSION

The patient had intractable grand mal seizures, severe psychomotor retardation and dysmorphic features. None of these findings were sufficient to encourage the clinicians to request a karyotype analysis until she was 8 years old. In reviewing the previously published cases with partial trisomy of the proximal long arm of chromosome 15, the main clinical features appeared to be mental retardation, short stature, microcephaly, hypotonia, seizure activity and certain dysmorphic features. The latter included widely spaced heavy eyebrows, telecanthus, epicanthus, strabismus, antimongoloid slant, low set, posteriorly rotated ears, large mouth, full lips and high arched palate. Scoliosis, mild syndactyly of the toes and clinodactyly of the 5th fingers were variable features. Nine patients including the present case had seizures of varying severity, including myoclonic and grand mal types. Our case shared most of these findings which in aggregate are rather nonspecific. However, the present case would suggest that karyotype analysis with different banding techniques be performed early in the evaluation of mental retardation associated with sei-

zure disorders and somatic dysmorphism. Results of karyotype analysis would be of potential diagnostic and counseling benefit.

This report also illustrates the difficulty encountered in the identification of a small extra chromosome with few landmarks in the absence of a parental chromosome abnormality. The proper diagnosis of the extra chromosome in this patient was achieved only after the employment of several banding techniques.

ACKNOWLEDGEMENTS

This work was supported in part by the Ranken Jordan Trust Fund for the Crippling Diseases of Children and by USPHS grant RR 00036 from the Division of Research Resources.

REFERENCES

- Centerwall W R & Morris J P. Partial D 15 trisomy. *Hum Hered* 25: 44, 1975.
- Cohen M M, Ornoy A, Rosenmann A & Kohn G. An inherited translocation t(4;15)(p16;q22) leading to two cases of partial trisomy 15. *Ann Genet* 18: 99, 1975.
- Crandall B F, Muller H M & Bass H W. Partial Trisomy of chromosome number 15 identified by trypsin Giemsa banding. *Am J Ment Def* 77: 571, 1973.
- Mankinen C B, Holt J G & Sears J W. Partial trisomy 15 in a young girl. *Clin Genet* 10: 77, 1976.
- Power M M, Barry R G, Cannon D E & Master son J G. Familial partial trisomy 15. *Ann Genet* 20: 159, 1977.
- Watson E J & Gordon E H. A case of partial trisomy 15. *J Med Genet* 11: 400, 1974.
- Webb G C, Carlson O M, Robson M K & Pitt D H. A partial D trisomy/normal mosaic female. *J Med Genet* 8: 52, 1971.
- Wurster Hill D H & Hoefnagel D. Banding identification of partial trisomy 15 and of 8;11 translocation. *J Ment Defic Res* 11: 139, 1974.

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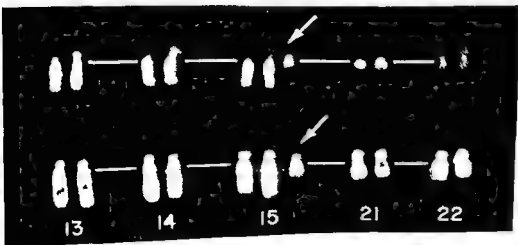
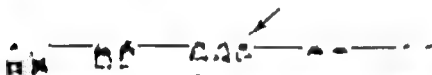


Fig. 2 In descending order: GTG, QFQ and RHA banded partial karyotypes of the patient with extra No. 15

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CASE REPORT

THE COFFIN SIRIS SYNDROME

A SCHINZEL

From the Department of Medical Genetics University of Zurich Zurich Switzerland

ABSTRACT Schinzel A (Department of Medical Genetics University of Zurich Switzerland) The Coffin Siris syndrome *Acta Paediatr Scand* 68 449, 1979 — A 14 month-old female with the Coffin Siris syndrome is described. Typical features included underweight at birth, growth retardation, microcephaly, profound mental retardation, severe hypotonia with lax joints, feeding difficulties and frequent respiratory tract infections, sparse scalp hair, small nose, epicanthic folds, a prominent philtrum and full lips, a congenital heart defect, hypoplasia or aplasia of the distal phalanges of digits 2-5 and the corresponding nails, especially of the fifth fingers and toes, and aplasia of the middle phalanges of the little fingers and the second and fifth toes, severe delay in bone maturation. The proposita also showed hypoplasia of the lateral portions of both clavicles. Inheritance of the Coffin Siris syndrome is possibly autosomal recessive.

KEY WORDS Coffin Siris syndrome, autosomal recessive inheritance, phalangeal hypoplasia.

In 1970 Coffin & Siris (3) described three unrelated patients with a distinct pattern of malformations and severe mental retardation. Subsequently only eight further cases have been reported (1, 2, 5, 6). The present paper reports a further case of the Coffin Siris syndrome.

CASE REPORT

Case history

The proposita was the product of the second pregnancy of a 31-year-old mother and a 30-year-old father. Family history was positive for diabetes mellitus and eczema; there was no consanguinity. The mother has never had any kind of epileptic episodes and never has taken any antiepileptic drugs. The 21-year-old son is healthy and normal. He was delivered by caesarean section because of a narrow pelvis of the mother; his birthweight was 3750 g.

During the entire pregnancy with the proposita, the mother suffered from abdominal pain and frequent vomiting. A one-day bleeding occurred in the 16th week. At the end of the sixth month, intrauterine growth retardation was first detected. Repeated human placental lactogen blood levels and estriol values in the urine of the mother were normal. At the 40th week of gestation, caesarean section was performed because of a pathologic cardiotocographic curve, breech presentation and previous section. The newborn was given artificial ventilation

with oxygen for the first ten minutes. Apgar scores were 6/9 and 9/9 after 1/5 and 10 minutes respectively.

Clinical examination at birth disclosed severe muscular hypotonia and virtually complete lack of spontaneous movements, short eyelids, a hoarse cry, a small anterior fontanel, redundant nuchal skinfolds, moderate hypertrichosis and hypoplasia or aplasia of the distal phalanges of the second through fifth fingers and toes. Measurements were 46 cm for length (10th percentile), 2430 g for weight and 31 cm for head circumference (both below 10th percentile). An ophthalmologic examination disclosed normal bulbs and fundi. The proband's neonatal course was marked by failure to thrive, poor sucking requiring intermittent tube feeding, repeated cyanotic and apneic spells during and after feeding, severe delay in psychomotor development, episodes of profuse sweating and frequent respiratory infections which necessitated five hospitalizations during the first 15 months of life. A systolic murmur with its maximum at the left sternal border was first noticed at five weeks of age. Neurologically, severe muscular hypotonia persisted. Moro and grasping reflexes were absent and spontaneous movements continued to be very poor as were tendon reflexes. She started to fix with her eyes for a short span at 3½ months of age. At that time, intermittent convergent squint and intermittent horizontal nystagmus were noticed. The first smile was observed at six months.

At clinical examination at 14 months of age, the proposita presented as a microcephalic and severely hypotonic, physically and mentally retarded female infant with multiple dysmorphic stigmata (Figs 1-7). Length (68.9 cm), weight (6.35 g) and head circumference (41 cm) were

CASE REPORT

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Table 1 Main findings in twelve cases of the Coffin Sirtis syndrome as compared to their incidence in the fetal hydantoin syndrome

	References					Present case	Fetal hydantoin syndrome (4)
	7	4	5	6	1		
Birthweight below 3rd percentile	6/10	—	—	—	—	+	13/14
Perinatal growth retardation	9/9	—	—	—	—	+	14/15
Micrencephaly	9/10	—	—	—	—	+	13/15
Mental retardation (IQ below 85)	9/9	+	+	+	+	+	9/13
Muscular hypotonia	10/10	+	+	+	+	+	0/15
Retarded bone maturation	5/6	—	—	—	—	+	Not mentioned
Feeding difficulties	9/10	—	—	—	—	+	n m
Recurrent respiratory infections	5/10	—	—	—	—	+	n m
Hypertriehosis	9/10	?	?	?	?	+	n m
Sparsely scalp hair	8/9	—	—	—	—	+	n m
Facial dysmorphism	10/10	+	+	+	+	+	6/15
Full lips	8/9	—	—	—	—	+	n m
Hypoplasia of terminal phalanges and nails	10/10	+	+	+	+	+	4/15
Hypertendible joints	10/10	+	+	+	+	+	n m
Cleft palate	7/10	—	—	—	—	—	1/15
Congenital heart defect	4/10	—	—	—	—	+	3/15
Dandy Walker malformation in autopsied cases	7/3	0	0	0	0	0	0

and the left index finger were absent and those of the other fingers except for the thumbs were hypoplastic most pronounced for the right index and the left middle finger (Fig. 1). This nail hypoplasia was associated with shortening of the distal phalanges of the corresponding fingers. The thumbs were mostly in flexion and opposition there was clinodactyly of the little fingers. The feet were in adduction and calcaneo-valgus position. The nails of the second and fifth toes were absent and the nails of the third and fourth toes were hypoplastic. Tendon reflexes were very weak. The proposita reacted to sounds and rarely smiled but she did not turn around or sit up. Grasping was very poor and she was barely able to lift her head. A Denver development test revealed a developmental age of 4-5 months.

The further course was marked by repeated respiratory infections which were accompanied with mild cardiac failure and persisting severe mental retardation. Presently at 4 1/2 years of age she is still unable to sit up and has no speech.

X-ray examinations

On chest films performed at 14 months of age (Fig. 3) the heart was enlarged to the left and lung vascularity was increased. A pneumonia was seen in the left lower lobe behind the heart. There was sinking hypoplasia and superiorly convex curving of the lateral portions of the clavicles. Radiographs of both hands at three days of age (Fig. 4) revealed no ossification of the distal phalanges of both second and fifth fingers and the middle phalanges of the little fingers and hypoplasia of the distal phalanges of the third and fourth fingers. At 14 months the findings were similar and bone age was less than three months. Radiographs of both feet at three days of age (Fig. 5) showed hindfoot valgus and absence or hypoplasia of the middle and distal phalanges of toes 3-5. Skull

and pelvic films at 14 months of age were considered normal.

Other examinations

An EKG showed right ventricular hypertrophy. In combination with the clinical findings and the chest films a



Fig. 5 Radiographs of both feet 3 days of age. No ossification of any distal phalanges except for the big toes nor of the middle phalanges of the second and fifth toes. Hypoplasia of the middle phalanges of the third and fourth toes.



Fig. 1 Head of the proposita aged 14 months. Sparse scalp hair, short nose with broad bridge, bilateral epicanthic folds, convergent squint of both eyes, prominent philtrum and lips.

below the 3rd percentile. Scalp hair was sparse. The facies was small and narrow and exhibited a broad and short nose, narrow palpebral fissures (on the left side more than on the right), bilateral epicanthal folds, convergent strabismus of both eyes, normal fundi, full phil-

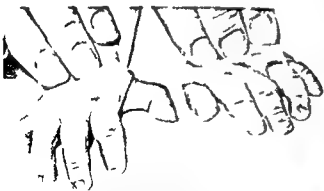


Fig. 2 Both hands of the proposita aged 14 months. Hypoplastic terminal phalanges of fingers 2-5 with absent nails on little fingers and hypoplastic nails on fingers 2-4 (especially both index fingers and the left middle finger). Clinodactyly of both little fingers.



Fig. 3 Chest films from the proposita at 14 months of age. Enlarged heart, increased lung vascularity, left lower lobe consolidation, and hypoplasia of the lateral portions of both clavicles.

trum and lips, a small mouth and a rather large plump tongue and a misshapen left ear. There was eczema over the nuchal area and a chronic eczema of the retroauricular, nuchal and dorsal upper thoracic regions and mild hypertrophicosis of the back and dorsal upper arms. Cardiac auscultation disclosed a 4/6 systolic murmur over the entire heart and the back; the liver was palpable 7.1 cm but the spleen was not palpable. The abdominal muscles were hypoplastic with diastasis of the rectus muscles. No hernias were present; genitalia were normal; there was a precoccygeal dimple. All large and small joints were hyperextensible. The nails of both little fingers



Fig. 4 Radiographs of both hands, 3 days of age. No ossification of distal phalanges of the second and fifth fingers and of middle phalanges of the fifth fingers; hypoplasia of distal phalanges of the third and fourth fingers.

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Postnatal growth retardation	9/9	—	+	+	—	+	14/15
Microcephaly	9/10	—	+	+	—	+	13/15
Mental retardation (IQ below 85)	9/9	+	+	+	—	+	9/13
Muscular hypotonia	10/10	+	+	+	—	+	0/15
Retarded bone maturation	5/6	—	+	+	—	+	Not mentioned
Feeding difficulties	9/10	—	+	+	—	+	n m
Recurrent respiratory infections	5/10	—	+	+	—	+	n m
Hypertichosis	9/10	+	+	+	—	+	n m
Spars scalp hair	8/9	+	+	+	—	+	n m
Facial dysmorphism	10/10	+	+	+	—	+	6/15
Full lips	8/9	—	+	+	—	+	n m
Hypoplasia of terminal phalanges and nails	10/10	+	+	+	—	+	4/15
Hypertendible joints	10/10	+	+	+	—	+	n m
Cleft palate	7/10	—	—	—	—	—	1/15
Congenital heart defect	4/10	—	+	+	—	+	3/15
Dandy Walker malformation in autopsied cases	1/3	0	0	0	0	0	0

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Fig 5 Radiographs of both feet 3 days of age. No ossification of any distal phalanges except for the big toes nor of the middle phalanges of the second and fifth toes. Hypoplasia of the middle phalanges of the third and fourth toes.

ventricular septal defect possibly combined with a mild pulmonic stenosis seemed most likely. No cardiac catheterization was performed.

Tests for thyroid function gave normal results. Toxoplasmosis titer was negative. A banded chromosome examination disclosed a normal 46 XX karyotype.

DISCUSSION

The proband is a characteristic case of the Coffin Sins syndrome (Table 1). She revealed all the pertinent features of this syndrome including pre and postnatal growth deficit, severe delay of bone maturation, microcephaly, muscular hypotonia, mental retardation, hypoplasia of distal phalanges and nails, sparse scalp hair, hypertichosis, repeated respiratory tract infections, and a characteristic facies with short and broad nose, epicanthic folds, and prominence of philtrum and lips. Less consistent findings of the Coffin Sins syndrome are congenital heart defects and cleft palate; the latter was not found in the proband. The Dandy Walker malformation was observed in the only two autopsied cases (1, 6). Hypoplasia of the distal portions of the clavicles has not been described in previous cases, but it may have been overlooked.

One case showed an incomplete clinical picture (1) (see Table 1). Presently, the knowledge of the variability of the clinical findings in the Coffin Sins syndrome is too small to allow to be sure that this case really represents the same syndrome.

The fetal hydantoin syndrome shares some clinical features with the Coffin Sins syndrome, notably distal limb hypoplasia, pre and postnatal growth retardation, microcephaly, mental retardation, cleft palate, and congenital heart defects (4) (see Table 1). However, differentiation of these two clinical entities by pregnancy history (intake of hydantoin) as well as clinical findings will generally not be difficult. In the Coffin Sins syndrome, in contrast to the fetal hydantoin syndrome, growth and mental retardation is usually more severe, scalp hair is sparse, and hypoplasia of the distal phalanges is most severe in the fifth and second digits and not found in the thumbs

and big toes. In addition, the pattern of facial dysmorphism is different in the fetal hydantoin syndrome: a prominent forehead, synphysis, depressed nasal bridge, and a strikingly short, upturned nose are the most outstanding features, and a prominent philtrum is sometimes found, whereas in the Coffin Sins syndrome, full lips and a prominent philtrum are predominant, and the nose is normal or slightly shortened.

Karyotypes have been normal in all cases so far reported (2, 3, 5, present report). Although there was no instance of parental consanguinity, occurrence of the Coffin Sins syndrome in two sibs of different sex (2) out of a total of twelve reported cases indicates that inheritance might be autosomal recessive.

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The author wishes to thank Prof. A. Giedion (Division of Pediatric Radiology, Department of Pediatrics, University of Zurich) for valuable comments and Prof. W. Schmid (Department of Medical Genetics, University of Zurich) for the karyotype examination and for a review of the manuscript.

REFERENCES

1. Bartsokas C S & Tsiantos A K. Mental retardation with absent fifth fingernail and terminal phalanx. *Am J Dis Child* 120: 493, 1970.
2. Carey J C & Hall B D. The Coffin Sins syndrome. *Am J Dis Child* 132: 667, 1978.
3. Coffin G & Sins E. Mental retardation with absent fifth fingernail and terminal phalanx. *Am J Dis Child* 119: 433, 1970.
4. Hanson J W, Mynanthopoulos N C, Sedwick Harvey M A & Smith D W. Risk to the offspring of women treated with hydantoin anticonvulsants with emphasis on the fetal hydantoin syndrome. *J Pediatr* 89: 662, 1976.
5. Tunnessen W W, McMillan J A & Levin M B. The Coffin Sins syndrome. *Am J Dis Child* 132: 393, 1978.
6. Weisswasser W H, Hall B D, Delavan G W & Smith D W. Coffin Sins syndrome. *Am J Dis Child* 125: 838, 1973.

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CASE REPORT

REGIONAL DEFICIENCY OF SECRETORY IgA IN A PATIENT WITH COMBINED IMMUNODEFICIENCY OF THE ADA DEFICIENT TYPE

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From the New York State Department of Health Birth Defects and Kidney Disease Institutes
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ABSTRACT Moore E C Laffin R J Tomasi T Pickering R J Radl J and Meuwissen H J (Department of Pediatrics Albany Medical College Albany New York New York State Department of Health Birth Defects and Kidney Disease Institutes Department of Immunology Mayo Medical and Graduate School Rochester Minnesota USA and Institute for Experimental Gerontology TNO Rijswijk The Netherlands) Regional deficiency of secretory IgA in a patient with combined immunodeficiency of the ADA deficient type *Acta Paediatr Scand* 68 453 1979 —The IgA system in a patient with SCID and ADA deficiency showed heterogeneity Serum IgA and stool secretory IgA (SIgA) levels were normal but with altered κ/λ and A₁/A₂ subclass ratios IgA in saliva and urine was deficient Amounts of secretory component were normal Jejunal and rectal biopsies showed prominent lymphonodular hyperplasia but no cells containing IgA A normal serum IgA level therefore does not always predict an intact secretory IgA system

KEY WORDS IgA combined immunodeficiency disease adenosine deaminase deficiency

In almost all patients reported the presence or absence of serum IgA has correlated well with the presence or absence of secretory IgA (SIgA). Only rare patients have either possessed secretory IgA without serum IgA (4 17) or had normal serum IgA with deficient saliva IgA (2 4 15 16).

Our patient had severe combined immune deficiency disease (SCID) with normal IgA levels in serum and stool but was markedly deficient in salivary and urinary IgA. Secretory component (SC) was readily detectable. She was the first patient found lacking adenosine deaminase (ADA) (1 5 6 7).

CASE REPORT

This child had persistent pneumonia and candida infections from the second week of life. After six months of age when the diagnosis of SCID was made she was

nursed in a low pathogen environment where despite persistent interstitial pulmonary disease and intermittent oral candidiasis she did quite well for the next 1½ years. Fetal thymus and liver transfer factor and maternal bluffy coat cells (washed and irradiated) produced no significant improvement.

There were no HLA compatible siblings but because the patient did not stimulate her mother's cells in mixed lymphocyte culture maternal marrow transplantation was attempted with re-establishment of B cell but not of T cell function. The patient concurrently developed active cytomegalovirus infection with dissemination and died (7).

METHODS

All studies unless so stated were prior to marrow transplantation.

Serum immunoglobulin (Ig) levels, κ and λ light chain typing, subclass typing and analysis for dimeric structure of serum IgA were performed according to published methods (3 11 17).

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ventricular septal defect possibly combined with a mild pulmonic stenosis seemed most likely. No cardiac catheterization was performed.

Tests for thyroid function gave normal results. Toxoplasmosis titer was negative. A banded chromosome examination disclosed a normal 46, XX karyotype.

DISCUSSION

The proposita is a characteristic case of the Coffin Sins syndrome (Table 1). She revealed all the pertinent features of this syndrome including pre- and postnatal growth deficit, severe delay of bone maturation, microcephaly, muscular hypotonia, mental retardation, hypoplasia of distal phalanges and nails, sparse scalp hair, hypertrichosis, repeated respiratory tract infections, and a characteristic facies with short and broad nose, epicanthic folds, and prominence of philtrum and lips. Less consistent findings of the Coffin Sins syndrome are congenital heart defects and cleft palate; the latter was not found in the proposita. The Dandy Walker malformation was observed in the only two autopsied cases (1, 6). Hypoplasia of the distal portions of the clavicles has not been described in previous cases, but it may have been overlooked.

One case showed an incomplete clinical picture (1) (see Table 1). Presently the knowledge of the variability of the clinical findings in the Coffin Sins syndrome is too small to allow to be sure that this case really represents the same syndrome.

The fetal hydantoin syndrome shares some clinical features with the Coffin Sins syndrome, notably distal limb hypoplasia, pre- and postnatal growth retardation, microcephaly, mental retardation, cleft palate, and congenital heart defects (4) (see Table 1). However, differentiation of these two clinical entities by pregnancy history (intake of hydantoin) as well as clinical findings will generally not be difficult. In the Coffin Sins syndrome, in contrast to the fetal hydantoin syndrome, growth and mental retardation is usually more severe, scalp hair is sparse, and hypoplasia of the distal phalanges is most severe in the fifth and second digits and not found in the thumbs

and big toes. In addition, the pattern of facial dysmorphisms is different in the fetal hydantoin syndrome: a prominent forehead, synophrys, depressed nasal bridge, and a strikingly short, upturned nose are the most outstanding features, and a prominent philtrum is sometimes found, whereas in the Coffin Sins syndrome, full lips and a prominent philtrum are predominant, and the nose is normal or slightly shortened.

Karyotypes have been normal in all cases so far reported (2, 3, 5, present report). Although there was no instance of parental consanguinity, occurrence of the Coffin Sins syndrome in two sibs of different sex (2) out of a total of twelve reported cases indicates that inheritance might be autosomal recessive.

ACKNOWLEDGEMENTS

The author wishes to thank Prof. A. Giedion (Division of Pediatric Radiology, Department of Pediatrics, University of Zurich) for valuable comments and Prof. W. Schmid (Department of Medical Genetics, University of Zurich) for the karyotype examination and for a review of the manuscript.

REFERENCES

1. Bartsokas C S & Tsiantos A K. Mental retardation with absent fifth fingernail and terminal phalanx. *Am J Dis Child* 120: 493, 1970.
2. Carey J C & Hall B D. The Coffin Sins syndrome. *Am J Dis Child* 132: 667, 1978.
3. Coffin G S & Sins E. Mental retardation with absent fifth fingernail and terminal phalanx. *Am J Dis Child* 119: 433, 1970.
4. Hanson J W, Mynanthopoulos N C, Sedwick Harvey M A & Smith D W. Risk to the offspring of women treated with hydantoin anticonvulsants with emphasis on the fetal hydantoin syndrome. *J Pediatr* 89: 662, 1976.
5. Tunnessen W W, McMillan J A & Levin M B. The Coffin Sins syndrome. *Am J Dis Child* 137: 393, 1978.
6. Weisswasser W H, Hall B D, Delavan E W & Smith D W. Coffin Sins syndrome. *Am J Dis Child* 125: 838, 1973.

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EVALUATION OF IgA IN SALIVA

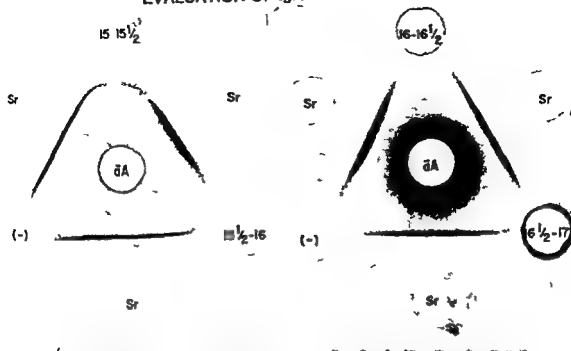


Fig 3 Double diffusion with anti IgA antisera (aA in center well) Sr Serum diluted to a concentration of IgA of 10^{-4} mg (-) blank well Other wells contain saliva

concentrated 40 times The numbers refer to the patient's age in months when the saliva was collected The decline in IgA levels with increasing age is evident

dominantly IgA κ type When marrow cells were stained with anti μ chain antibody tagged with fluorescein isothiocyanate (FITC) in combination with anti κ chain antibody tagged with tetramethylrhodamine isothiocyanate (TRITC) 14% positive cells were found of which nine were also positive for κ When stained with anti- α chain FITC in combination with anti λ chain TRITC 16% positive cells were found none positive for λ

By double immunodiffusion IgA could be identified in saliva in trace amounts only when the saliva was concentrated at least 40 times and only before the 17th month of life (Figs 2 and 3) By radio immuno assay saliva IgA levels were less than 0.001 mg/dl (various samples) There was not enough material available to determine if this IgA was SIgA SC appeared quantitatively normal on double immunodiffusion Traces of IgG in saliva were also found during this period but IgM was not

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Radiologic study of the gut in the 15th month showed numerous small round filling defects jejunal and rectal biopsies showed these to represent lymphonodular aggregates Immunofluorescent staining of the specimens

SERUM IMMUNOGLOBULINS

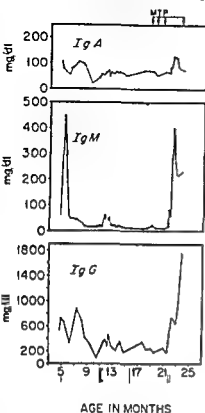


Fig. 1 Immunoglobulin levels versus age. Administration of gamma globulin or plasma is indicated by the vertical lines along the abscissa. Arrows indicate marrow transplantation (MTP). The clear areas indicate the range of normal. The IgM peak in the 5th month followed a series of immunizations (DPT, typhoid).

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the original volume of the specimen. Control samples were from normal children and patients of similar age with non infectious and non gastroenterological illnesses.

Urine samples pooled by time intervals were centrifuged and the supernatant was concentrated by filtration through a collodion bag retaining substances >25000 daltons.

IgG and CS in stool extracts and in saliva concentrated to more than 100 fold were studied by double diffusion using mono specific antisera (Nordic Hyland). IgA levels in urine, saliva and stool extracts were measured by radio immuno assay technique (8, 9).

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	Immunoglobulin					
	γ	α	μ	δ	κ	λ
Patient	4	21	1	0	19	4
Normal	122 ^b (34-252)	109 (47-260)	60 (45-117)	0.9 (0-21)		1.65 0.81-2.44

Number of Ig containing cells per 10^3 immunocytes (lymphocytes or plasma cells)
^b Geometric mean plus range

EVALUATION OF IgA IN SALIVA

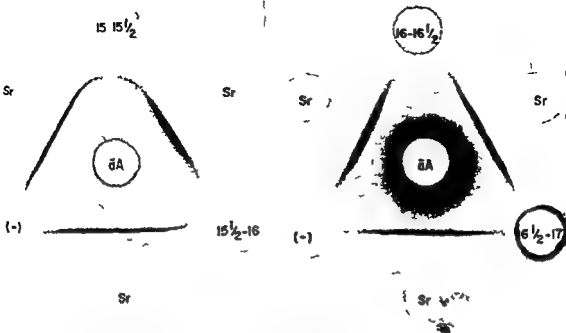


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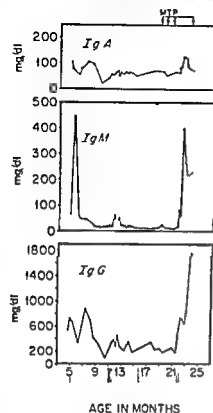


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EVALUATION OF IgA IN SALIVA

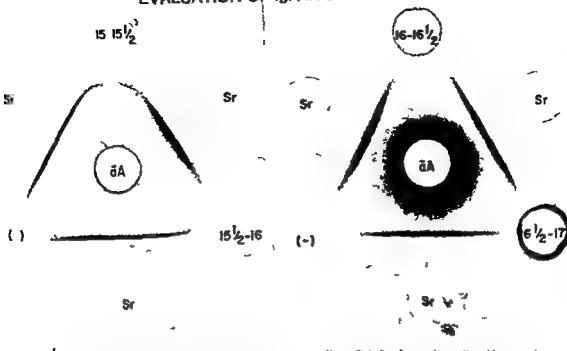


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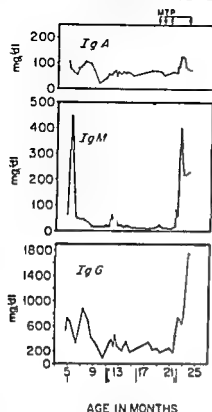


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EVALUATION OF IgA IN SALIVA

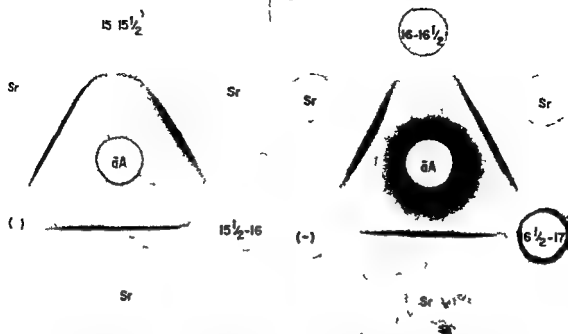


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IMMUNOELECTROPHORESIS OF SALIVA AND STOOL EXTRACTS

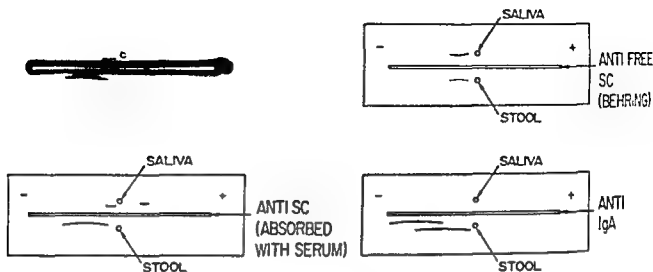


Fig. 3 Left side (photograph and diagram) shows the reaction of patient saliva and stool with an anti SC antiserum capable of reacting with SC either free or bound to IgA (anti SC (Nordic)). Similar analysis is shown diagrammatically on the right with an anti SC antiserum capable of reacting with free SC (anti SC (Behring)) (top) and with an anti IgA antiserum (bottom). Patient saliva shows an identical reaction with both SC antisera and no reaction with anti IgA indicating all the detectable SC is free. Stool extract shows a similar reaction with the anti

IgA and the anti SC (Nordic) but only a trace reaction to the anti SC (Behring) indicating that most though not all of the detectable SC is associated with IgA i.e. is SIgA. Additional precipitation lines seen in some instances may represent split products or cross reacting materials in enteric contents. Simultaneous studies done with substances containing known IgA, SIgA and SC confirmed their migration patterns under the conditions of the experiment.

showed no IgA containing cells but did reveal a few IgM and a very few IgG containing cells. At autopsy these lymphoid nodules were no longer found.

Radioimmunoassay capable of detecting as little as 0.015 mg/dl showed no IgA in urine samples concentrated 30–40 times.

The numerous lymph nodes found at autopsy contained many pyroninophilic cells, plasma cells and a few germinal centers depleted of lymphocytes. Some of the plasma cells were fluorescent for IgG and IgM but not for IgA. Intestinal tissues at autopsy also showed no IgA containing cells.

DISCUSSION

Our patient had normal levels of monomeric serum IgA of restricted heterogeneity, largely IgA κ and of subclass A1. Serum IgM and

IgG decreased to subnormal levels during the early months of life. Many children with ADA negative SCID lack one or more classes of Ig; the reason for this is unclear. The decline of IgG and IgM is consistent with the immunologic regression often seen in SCID, particularly the ADA negative type. The only site where IgA containing cells were found was the marrow; it therefore seems likely that most if not all of the serum IgA was produced there.

Little or no IgA was found in urine or saliva. It is unlikely that salivary IgA was destroyed by proteolytic cleavage; this has not happened in saliva samples preserved frozen for several years by one of us (J.R.) furthermore lip biopsy showed no IgA containing cells. Therefore despite normal levels of serum IgA, urinary and salivary IgA can be absent or extremely low.

During life and at autopsy samples of intestinal mucosa showed no IgA containing cells nevertheless in stool IgA was present at least partly bound to SC. Our sampling may have missed the IgA containing cells though we consider this unlikely; alternatively transudated serum IgA may have become attached to SC. Finally dimeric IgA produced in the marrow may have been transported in plasma and secreted into the gut as occurs in normal individuals (12, 13). Our failure to detect dimeric IgA in the serum does not negate this latter possibility as dimeric IgA in serum is normally present in very small amounts requiring special techniques for detection and may have been present at levels below the sensitivity of our tests.

Our patient is the first with SCID in whom lymphonodular hyperplasia of the gut has been described. This condition may be found in normal subjects but is most often seen with hypogammaglobulinemia or dysgammaglobulinemia. The functional significance of this abnormality and its relation to B cell development, the SIgA system and ADA remains unknown.

Other patients have been described with partial deficiencies in their IgA systems. Rubinstein et al. described a boy with SCID who had normal IgA levels in serum, jejunal secretions and stool but no detectable IgA in saliva, urine and tears (15). Studies for SIgA were not done and his ADA status is unknown. Strober et al. described a 15-year-old boy with normal levels of serum IgA, very low levels of IgA and undetectable SC in intestinal secretions and saliva; his IgA abnormalities were thought secondary to the SC deficiency (16). Krakauer et al. described an adult who died with renal failure and severe malabsorption who had normal serum IgA levels and markedly decreased SIgA in secretions (2).

Our current concepts of IgA, SIgA and B cell development do not adequately explain these discrepancies between serum IgA and SIgA. Unknown factors possibly related to

ADA function appear to be capable of influencing the development of B cell immunity to produce compartmentalization of the IgA system.

ACKNOWLEDGMENTS

We thank Drs J. Vossen and G. Scherer for immunofluorescent studies and I. H. Porter for critical review of the paper. Ms E. Schreinemackers and M. Nulb gave technical and secretarial assistance respectively.

REFERENCES

- Giblett E R, Anderson J E, Cohen F, Pollara B & Meuwissen H J. Adenosine deaminase deficiency in two patients with severely impaired cellular immunity. *Lancet* II 1067, 1972.
- Krakauer M, Zinneman H H & Hong R. Deficiency of secretory IgA and intestinal malabsorption. *Am J Gastroenterol* 64 319, 1975.
- Mancini G, Carbonara A O & Meremona J F. Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry* 2 735, 1965.
- Mestecky J & Lawton A. Report of the International Symposium on the Immunoglobulin A System. Birmingham, Alabama, 1973. In *Advances in experimental medicine and biology*, Plenum Press, New York and London, 1974, vol. 45, p. 446.
- Meuwissen H J, Pickering R J, Pollara B & Porter I H. Combined immunodeficiency disease and adenosine deaminase deficiency. A molecular defect. Academic Press, New York, 1975.
- Meuwissen H J, Pollara B & Pickering R J. Combined immunodeficiency disease associated with ADA deficiency. *J Pediatr* 86 169, 1975.
- Morre E C, Pollara M, Propp R & Meuwissen H J. Combined immunodeficiency of the ADA deficient type with reconstitution of B cell function after maternal marrow graft. To be published in the report of the Second Albany Conference on ADA Deficiency.
- Morgan C & Lazarow A. Immunoassay of insulin using a two antibody system. *Proc Soc Exp Biol Med* 110 79, 1967.
- Morrison M, Boyse G & Webster H. Use of lactoperoxidase catalyzed iodination in immunochemical studies. *Immunochemistry* 8 89, 1971.
- Ortega L G & Mellors R C. The role of localized antibodies in the pathogenesis of nephrotic nephrosis in the rat. *J Exp Med* 104 151, 1956.
- Radi J. Light chain typing of immunoglobulins in small samples of biological material. *Immunology* 19 137, 1970.
- Radi J, Schult H R E, Mestecky J & Hymans W. Report of the International Symposium on the Immunoglobulin A System. Birmingham, Alabama, 1973. In J. Mestecky & A. Lawton (eds), *Advances in ex*

- perimental medicine and biology* Plenum Press New York and London 1974 vol 45 p 57
- 13 Radl J Swart A C W & Mestecky J The nature of the polymeric serum IgA in man *Proc Soc Exp Biol Med* 150 482 1975
 - 14 Radl J Vandenberg P & Snooyink J J Antisera specific for human polymeric IgA *In H Ecters (ed) Protides of the biological fluids* Pergamon Press Oxford New York 1977
 - 15 Rubinstein A Radl J Cotter H Rossie I & Gugler F Unusual combined immunodeficiency syndrome exhibiting kappa IgD paraproteinemia residual gut immunity and graft versus host reaction after plasma infusion *Acta Paediatr Scand* 62 365 1973
 - 16 Strober W Krakauer H Klaeveman H I Rey nolds H Y & Nelson D L Secretory component deficiency A disorder of the IgA immune system *N Engl J Med* 294 351 1976
 - 17 Swinson V Dyce B Citron P Rouleau C Feinstein D & Haverack B J Absence of IgA in serum with presence of IgA containing cells in intestinal tract *Clin Res* 16 119 1968 (Abstract)
 - III Vossen J *The development of the B immune system in man* Thesis University of Leiden 1975

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CASE REPORT

A VIRILIZING ADRENAL TUMOR WITH BORDERLINE ELEVATION OF URINARY 17 KETOSTEROIDS AND HISTOCHEMICAL DEMONSTRATION OF A DEFICIENCY IN THE $\Delta 5/\Delta 4$ ISOMERASE 3β HYDROXYSTEROID DEHYDROGENASE ENZYMATIC SYSTEMM MONTELEONE NETO¹ J A MELLO DE OLIVEIRA² M F S SA³ and J CORNICELLI*From the Departments of ¹Genetics ²Pathology ³Gynecology and Obstetrics and Surgery Faculty of Medicine of Ribeirão Preto University (São Paulo) S P Brazil*

ABSTRACT Monteleone Neto M Mello de Oliveira J A Sa M F S and Cornicelli J (Departments of Genetics Pathology Gynecology and Obstetrics and Surgery Faculty of Medicine Ribeirão Preto Brazil) A virilizing adrenal tumor with borderline elevation of urinary 17 ketosteroids and histochemical demonstration of a deficiency in the $\Delta 5/\Delta 4$ isomerase 3β hydroxysteroid dehydrogenase enzymatic system *Acta Paediatr Scand* 68 459 1979 —A 3-year-old girl affected by a virilizing tumor of the adrenal gland without significant elevation in the levels of 17 ketosteroids (17 KS) urinary excretion was studied clinically. Her symptoms started abruptly at the age of 2 with progressive enlargement of the clitoris and the appearance of pubic hair. In various tests the 17 KS levels barely exceeded the upper normal limits and at times remained within normal limits. The retroperitoneum X ray suggested an enlargement of the right adrenal gland and the presence of a neoplasm which was actually discovered during surgery. Histopathological examination revealed a well defined neoplasm without capsule invasion and with accentuated cell polymorphism. Histo-enzymology showed that the tissue lacked the enzymatic system involving 3β hydroxysteroid dehydrogenase (3β HSD). Indoxylase (IEST A) activity identified the tumor as originating from the internal layers of the adrenal cortex. The histochemical findings were correlated with the clinical picture and the levels of urinary 17 KS.

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Virilizing adrenal tumors frequently offer a characteristic clinical picture with a great increase in the urinary 17 KS levels and marked virilization. Virilization associated with urinary 17 KS levels close to upper limit of normal is a rare occurrence (10, 16, 19). It has been suggested that in such cases an enzymatic blockage occurs in the tumor tissue due to the absence or reduced activity of one of the enzymes involved in steroidogenesis (2, 5).

This paper describes a patient with an adrenal tumor which had virilizing effects but not accompanied by a marked elevation in urinary 17 KS. An enzymatic defect in the synthesis of adrenocortical steroids was demonstrated.

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A 3-year-old white girl (R S P Reg 141778) was admitted to the hospital with a history of pubic hair growth and increased size of the clitoris for a period of one year. During the same period of time the child's weight and height increased dramatically. Her behaviour was aggressive and she was irritable but her general health was good. On examination her height was 103 cm and weight 15.7 kg. She had well developed subcutaneous and muscular tissue. She had no acne or any axillary hair. There was hair in the pubic and vulvar regions. The labia majora were hypertrophic with skin rugosity. The labia minora were hyperpigmented and hypertrophic. The clitoris was peniform 5 cm long with hypertrophic cavernous bodies present (Fig. 1). The urethral meatus was located in a

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- perimental medicine and biology*. Plenum Press, New York and London 1974 vol 45 p 57
- 13 Ridi J, Swirt A C W & Mestecky J. The nature of the polymeric serum IgA in man. *Proc Soc Exp Biol Med* 150 482 1975
 - 14 Ridi J, Vandenberg P & Snooyink J J. Antiserum specific for human polymeric IgA. In H. Pieters (ed.) *Protides of the biological fluids*. Pergamon Press, Oxford, New York 1977
 - 15 Rubinstein A, Ridi J, Cottier H, Rossie F & Gugler E. Unusual combined immunodeficiency syndrome exhibiting kappa IgD paraproteinemia, residual gut immunity and graft versus host reaction after plasma infusion. *Acta Paediatr Scand* 62 365 1973
 - 16 Strober W, Krikauer R, Khaveman H I, Reynolds H Y & Nelson D I. Secretory component deficiency. A disorder of the IgA immune system. *N Engl J Med* 294 351 1976
 - 17 Swanson V, Dyce B, Citron P, Rouleau C, Feinstein D & Haverack B J. Absence of IgA in serum with presence of IgA-containing cells in intestinal tract. *Clin Res* 16 119 1968 (Abstract)
 - 18 Vossen J. *The development of the B immune system in man*. Thesis, University of Leiden 1975

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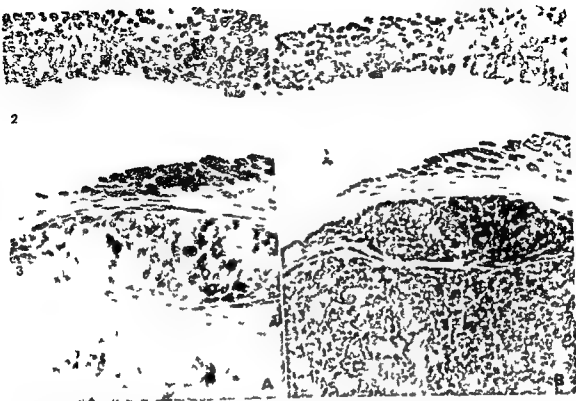


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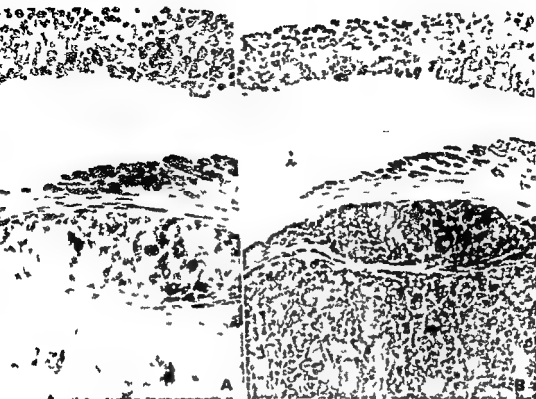


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of 3β HSD when dehydroepiandrosterone is utilized as a substrate is weaker in the reticular zone thus confirming the results of Goldman et al (5) for children of this age. In our case however the reaction intensity in this layer is not uniform showing focal points of activity very close to that of the external layers. The validity of a histochemical technique for the demonstration of 3β HSD activity in human adrenal cortex was also studied by Goldman et al (5) who showed the parallelism between results obtained by biochemical and histochemical techniques. The tumor that developed in the cortex did not show the cortical type of activity in the overwhelming majority of its cell population. Consequently this neoplasm acted as an independent gland incapable of synthesizing adequate adrenocortical steroids and carrying out an anomalous biosynthetic pathway characterized by $\Delta 5$ compounds. In such a biosynthetic pathway there are androgenic compounds capable of producing some virilization. One such instance occurs in the congenital adrenal Bongiovanni type hyperplasia in which there is a lack of activity of 3β HSD with virilization of external female genitalia and urinary excretion of $\Delta 5 3\beta$ ol compounds (1) but with high levels of urinary 17 KS suggesting that in tumor tissues another kind of hormone may possibly have been produced or another enzyme could be deficient too.

The advanced bone age of the present case could indicate increased DHA production. This possibility however could not be confirmed since no measurements were made of blood or urinary levels of DHA and other compounds of the steroidogenic pathway. The nearby non neoplastic tissue which showed a positive reaction for 3β HSD activity also acted as a control for the reaction of the tumor tissue. This demonstrates that the latter was unable to carry out C_3 oxidation in the cyclopentanophenanthrene ring thus confirming the hypothesis advanced by David et al (2). Goldman et al (5) demonstrated this same blockage in cases of hyperplasias ade-

nomas and carcinomas of the adrenal gland in which the high excretion of $\Delta 5 3\beta$ HSD products gave rise to the hypothesis of a decrease in the activity of 3β HSD which was then confirmed both histochemically and biochemically. In agreement Hoch Ligeti (11) also found a positive reaction for 3β HSD in non virilizing adrenal adenomas.

The oxidation reduction of C_3 is carried out by an enzymatic system involving actual C_3 oxidation reduction and isomerization of cyclopentanophenanthrene nucleus from its $\Delta 5$ to its $\Delta 4$ form in which a specific isomerase takes part. The histochemical method applied utilizes DHA as a substrate which is a steroid compound with a hydroxyl group in C_3 position and a double bond between C_3 and C_4 i.e. a 3β hydroxy $\Delta 5$ compound. Thus the final result of the reaction demonstrates the joint action of oxidoreductase and isomerase. The absence of formazan the final product of nitro blue tetrazolium reduction and the indicator of reaction intensity in the neoplastic tissue does not permit us to distinguish between the lack of one or the other of the enzymes involved.

In the present case we found that the I-EST-A reaction was positive in the non neoplastic cortical cells starting from the fascicular zone of the cortex and increasing in activity towards the reticular zone. The presence of the same enzymatic activity in the neoplastic tissue shows a differentiation in function for the tumor cells which is identical to that for the internal layers of the adrenal cortex and suggests that this neoplasm originates in the innermost cells of the cortical layer. I-EST-A activity is interpreted as an enzymatic activity connected with lipid metabolism. The present study shows that this activity involves enzymes other than the esterases which hydrolyze Tween 80 (T-EST-A) and naphthol acetate (N-EST-A) since the results of the three reactions are completely independent from the point of view of both intensity and topography.

The histochemical data allows us to con-

Table 1 *Histoenzymology of a virilizing neoplasm of the adrenal cortex and of the neighbouring non neoplastic tissue*

Enzymatic activity scale: n=negative ++=weak reaction +++=moderate reaction ++++=strong reaction ++++=very intense reaction tumor=neoplastic tissue Glom=glomerular Fascic=fascicular Retic=reticular MPS=mucopolysaccharides Med=medulla For further details see text

Metabolic pathway	Reaction	Tumor	Normal cortex			Med	Technique
			Glom	Fascic	Retic		
Pentose pathway	G6P D	++	++++	+++	+++	+	Wegman et al (71)
Pentose pathway	6PG D	++	++	+	+	n	Wegman et al (71)
Pentose pathway	NADH ₂ TR	++++	++++	+++	++++	+	Scarpelli et al (17)
Steroidogenesis	A DH	++	++	+	+	n	Hardonk (9)
Steroidogenesis	3 β HSD	n	+++	++	++	n	Levy et al (13)
Glycolysis	A DI	+	++	+	+	n	Hardonk (9)
Glycolysis	L D	+++	++	++	++	++	Nachlas et al (15)
Krebs cycle	M D	++	+++	++	++	++	Nachlas et al (15)
Krebs cycle	S D	+/++	+	+	+	+	Wegman et al (10)
Krebs cycle	NADH ₂ TR	++++	++++	+++	+++	++	Scarpelli et al (11)
Lipid metabolism	T I S T A	n	n	n	n	n	Gomon (8)
Lipid metabolism	I I S T A	+++	n	+	+++	n	Holt (17)
Lipid metabolism	N F S T A	+	+	+	+	n	Nachlas et al (14)
Lipid metabolism	α GI A I	n	n	n	n	n	Gomon (6)
Lipid metabolism	α GP A II	+	+	+	+	+	Gomon (7)
Amino acid metabolism	MAO	++	+	+	+	+	Glenner et al (4)
M P S metabolism	β GL A	n	n	n	n	n	Seligman et al (19)

In this reaction there are a few isolated cells showing enzymatic activity

activity was also found in the neoplasm and enzymes that are inactive in normal cortex were also inactive in the neoplasm

DISCUSSION

A few cases of female patients affected by virilizing tumors of the adrenal gland not associated with elevated levels of urinary 17 KS have been described in the literature (10-16, 19). This type of unusual association makes clinical diagnosis more difficult. In the usual cases associated with high levels of urinary 17 KS the adrenal suppression test confirms the diagnosis since after the administration of suppressive drugs there is no reduction of its excretory levels (23).

Some clinical signs might be present which will help make the diagnosis and indicate the necessity for surgical treatment. These are postnatal onset of progressive and rapid virilization, exaggerated growth of the clitoris and accelerated bone maturation. All of these signs were present in this patient who started show-

ing clitoral hypertrophy at 2 years of age and had a bone age of approximately 6 years at a chronological age of 3 years.

The lack of increase in the levels of urinary 17 KS in such cases has been the object of various speculations, one of which is the hypothesis advanced by David et al (2). They suggest that both the low 17 KS levels and the elevation in blood dehydroepiandrosterone (DHA) sulphate described in these cases could be explained by an enzymatic error in the tumor tissue. This enzymatic error would divert normal steroidogenesis towards the biosynthesis of androgenic non keto hormones or supply a different kind of steroid that are converted in peripheral tissues to more potent androgenic non keto hormones. According to David et al (2) 3 β HSD could be the deficient enzyme.

In our study we assessed the activity of various enzymes including 3 β HSD by histochemical techniques. The normal cortex has strong activity in every layer, intensity being highest in the glomerular tissue. The activity

of 3β HSD when dehydroepiandrosterone is utilized as a substrate is weaker in the reticular zone thus confirming the results of Goldman et al (5) for children of this age. In our case however the reaction intensity in this layer is not uniform showing focal points of activity very close to that of the external layers. The validity of a histochemical technique for the demonstration of 3β HSD activity in human adrenal cortex was also studied by Goldman et al (5) who showed the parallelism between results obtained by biochemical and histochemical techniques. The tumor that developed in the cortex did not show the cortical type of activity in the overwhelming majority of its cell population. Consequently this neoplasm acted as an independent gland incapable of synthesizing adequate adrenocortical steroids and carrying out an anomalous biosynthetic pathway characterized by $\Delta 5$ compounds. In such a biosynthetic pathway there are androgenic compounds capable of producing some virilization. One such instance occurs in the congenital adrenal Bongiovanni type hyperplasia in which there is a lack of activity of 3β HSD with virilization of external female genitalia and urinary excretion of $\Delta 5 3\beta$ ol compounds (1) but with high levels of urinary 17 K.S. suggesting that in tumor tissues another kind of hormone may possibly have been produced or another enzyme could be deficient too.

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Lipid metabolism	T FST A	n	n	n	n	n	Gomon (8)
Lipid metabolism	J FST A	+++	n	++	+++	n	Holt (17)
Lipid metabolism	N FST A	+	+	+	+	n	Nachlas et al (14)
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BOOK REVIEWS

D B Jelliffe & E F G Jelliffe *Human milk in the modern world* Oxford University Press 1978 500 pp illus £15.00 ISBN 0-19-64919-1

During the last few years breast feeding has become very much in the focus of interest. Every major meeting of paediatricians or nutrition scientists has breast feeding and breast milk on the agenda. Although breast milk is as old as mankind, new constituents are still being discovered, such as the water soluble Vitamin D and the many anti-infective factors. At present more than 100 components of breast milk are known.

The newly awakened interest has been further enhanced by the recent greatly publicized court case brought about by Nestle against the action group 'Arbeitsgruppe der dritten Welt' for distributing a pamphlet entitled 'Nestle kills babies'. This case has emphasized the extreme importance of prolonged breast feeding in poor and insalubrious conditions, particularly in developing countries. However, the subject of breast feeding in young child nutrition as part of the wider field of human food science has but a very modest share in the curricula of medical and nursing training. This has been made very clear by the WHO Collaborative Breast Feeding Study, soon to be published, which deals partly with the training of health personnel. Part of the problem, and indicating the low priority of the subject until recently, is the paucity of manuals and handbooks on breast feeding and breast milk. An encouraging example has been set by the Swedish Board of Health and Welfare, which has edited a factual, unbiased manual on breast feeding and breast milk for health workers.

Breast feeding is firmly associated with Demick and Patricia Jelliffe and vice versa. Over the years this inquisitive couple has written numerous scientific and popular articles on different aspects of the subject. Among other things, they have been very actively engaged in the fight against unethical marketing methods of the multinational baby food companies.

The Jelliffes have now collected their considerable knowledge in an impressive volume of 500 pages entitled *Human Milk in the Modern World*. Psychosocial, nutritional and economic significance. They have intended it for health professionals, nutritionists, community workers and national planners, as well as for mothers' groups.

The book is based on a very extensive survey of the literature—the bibliography comprises close to 1500 references and an additional last minute bibliography on other 100. Seemingly every aspect of the subject is elucidated and in considerable detail, including an interesting introductory chapter on 'Mammalian antecedents and adaptive suckling'. There we learn, for instance, that the blue whale has breast milk containing 50% fat to ensure a rapidly built up insulating fat layer in the young. Also that the kangaroo is born sized at birth (140 mg) but becomes firmly attached to the nipple in the pouch until mature, its breast milk becomes less concentrated as the embryo

grows and does not contain lactose. If the young kangaroo is brought up on cow's milk it develops cataract just like the human baby with galactosaemia!

A large number of appropriate introductory quotations ranging from the Koran to modern thinkers and nursery rhymes make fascinating additions to the otherwise highly interesting chapters. The biological and biochemical aspects are very proficiently elucidated from all angles and with all available up-to-date knowledge. The Mother/Infant Dyad and particularly the importance of not separating the mother and child at 'artificial' maternities is rightly pointed out. Breast feeding will benefit greatly both in the short and long run from initiation of suckling within a few minutes or hours. The worldwide economics of breast feeding, including the consequences of early weaning, are dealt with in great detail and interesting reviews are made of trends and influences in technological urban societies as well as in non-western countries. (Incidentally the term 'industrialized' would seem more correct than 'Western' in these connections.)

The Codes of Ethics recently published by the Ross Laboratories and by the ICIFI (ICIFI=International Council of Infant Food Industries) are given in the appendices with rather sarcastic criticism, but no alternatives are offered or discussed.

In the chapter on 'Practical programmes' only one page is devoted to 'Formula for use when medically indicated' for mothers unable or unwilling to breast feed. No recipes or formulas are given for the benefit of the health worker who has to advise the mother in severe ill health or mothers working out of the home in paid employment where Westernized culture attitudes and facilities do not permit nursing. The pendulum has swung from no-breast feeding to no-formula text books. The need for a realistic balance should, in my opinion, be given greater emphasis.

In 500 pages repetition and overlapping will inevitably occur and this book is no exception. But page references are abundant for the benefit of the one-chapter reader.

Human Milk in the Modern World is a major and commendable achievement, although at times somewhat biased and unrealistic in its non-acceptance of alternatives even when and where other forms of feeding (say between 3 and 6 months) are necessary for psychological or biological reasons or for reasons of social obligation. It will most certainly become a classic and should be read by all those for whom it is intended.

Yngve Hofvander

P B Beeson and D A Bass *The cosmophil* Holt Saunders Ltd Eastbourne 1978 £10.00 ISBN 0-7116-1650-X

Ever since Paul Ehrlich eighty years ago noticed the presence of a white blood cell with a peculiar affinity

clude that it is in adrenocortical functioning neoplasm originating in the internal layers of the adrenal cortex and bearing an enzymatic steroidogenesis defect which favors the formation of androgenic steroids. Even though such steroids might be related to the associated virilizing symptoms, it cannot be excluded on the basis of the results obtained that the hormones liberated by the tumor may have undergone further chemical transformations outside the adrenal gland (e.g. in the liver) leading to more powerful virilizing hormones.

REFERENCES

- Bongiovanni A M. Adrenogenital syndrome with deficiency of β hydroxysteroid dehydrogenase. *J Clin Invest* 41: 2086, 1967.
- David R, Auffret G & Gaudy H M. A virilizing adrenal tumor with borderline elevation of urinary 17 ketosteroids. *Pediatrics* 42: 139, 1968.
- Dreker I J, Heisler A, Seism G R, Stern S, Person S & McGavack T H. Determination of urinary steroids. I. Preparation of pigment free extracts and simplified procedure for estimation of total 17 ketosteroids. *J Clin Endocrinol* 17: 55, 1952.
- Glenner G G, Burnier H G & Brow G W. The histochemical demonstration of monoamine oxidase activity by tetrazolium salts. *J Histochem Cytochem* 55: 591, 1957.
- Goldman A M, Bongiovanni A M, Yakowic W C & Prader A. Study of Δ^5 β hydroxysteroid dehydrogenase in normal hyperplastic and neoplastic adrenal cortical tissue. *J Clin Endocrinol* 24: 994, 1964.
- Gomon G. The distribution of phosphatase in normal organs and tissues. *J Cell Comp Physiol* 17: 71, 1941.
- Gomon G. Distribution of acid phosphatase in the tissue under normal and pathological conditions. *Arch Pathol* 32: 189, 1947.
- Gomon G. *Microscopic histochemistry*. University of Chicago Press, Chicago, Ill, 1952.
- Hirshon K M J. A new method for the histochemical demonstration of steroid producing cells in human tissues. *Histochem* 5: 234, 1965.
- Hayles A B, Hahn H M Jr, Sprague R G, Bahay M C & Priestley J T. Hormone secreting tumors of the adrenal cortex in children. *Pediatrics* 37: 19, 1966.
- Hoch Ligeti C. Δ^5 β hydroxysteroid dehydrogenase activity in human adrenal cortical adenomas. *Arch Pathol* 93: 467, 1977.
- Holt S J. The value of fundamental studies of staining reactions in enzyme histochemistry with reference to indoxyl methods for esterase. *J Histochem Cytochem* 4: 541, 1956.
- Levy H, Deane H W & Rubin B L. Visualization of steroid β OH dehydrogenase activity in tissues of intact and hypophysectomized rats. *Endocrinology* 65: 932, 1959.
- Nachlis M M & Seligman A M. The histochemical demonstration of esterase. *J Natl Can Inst* 9: 415, 1949.
- Nachlis M M, Walker D G & Seligman A M. A histochemical method for the demonstration of diphosphopyridine nucleotide diaphorase. *J Biophys Biochem Cytol* 4: 29, 1958.
- Siez J M & Migeon C J. Studies of androgen in patients with adrenocortical tumors. *J Clin Endocrinol* 27: 617, 1967.
- Scarpelli D G, Hess H & Pearse A G H. The cytochemical localization of oxidative enzymes. I. Diphosphopyridine nucleotide diaphorase and triphosphopyridine nucleotide diaphorase. *J Biophys Biochem Cytol* 4: 747, 1958.
- Seligman A M, Tsou K E, Rutenberg S H & Cohen H H. Histochemical demonstration of β D-glucuronidase with a synthetic substrate. *J Histochem Cytochem* 2: 709, 1954.
- Toledo S P A, Coelho-Neto A S, Assis L M & Ulhoa Cintra A B. Tumor virilizante de supra-renal com elevação limitrofe dos níveis de 17-cetosteroides urinários. *Rev Ass Med Bras* 19: 71, 1971.
- Wegman R & Tordet Candroit C. Histochemical demonstration of succinic dehydrogenase by specific inhibition and activation. *J Histochem Cytochem* 9: 345, 1960.
- Wegman R & Gerzeli G. La glucose 6-phosphate dehydrogenase et ses corrélations avec des substrats voisins du glucose 6 phosphate. Role de l'hexokinase. *Ann Histochim* 6: 111, 1961.
- Wegman R & Sotelo C. Aspects cytoenzymologiques du métabolisme des glucides de la cellule de Purkinje. *Ann Histochim* 7H: 65, 1967.
- Wilkins L. *Diagnóstico y tratamiento de las enfermedades endocrinas en la infancia y la adolescencia*. Espiraco, Barcelona, 1966.

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this they are well known for creativity and good results. In one part of the book the situation of the nurses is described.

There are two appendices and the first one 'Scenes in Ward 7' could very well be a first independent chapter. Here Maureen Oswin describes everyday situations from the different wards in a dramatic and touching way. Here you find things that the evening press could easily distort. Maureen Oswin's description is objective and although the contents of the book as a whole is critical, every chapter ends with constructive recommendations.

There is a list of recommended further reading. It is good but publications of James and Joyce Robertson are missing.

The book is good reading for everybody who has to do with mentally handicapped children (medical consultants, psychologists, teachers, nurses, administrators etc). The book can be highly recommended and should be available in every hospital library.

Peter Vekås

E. L. Kendig Jr & V. Chernick (eds.) *Disorders of the respiratory tract in children*. 3rd ed. W. B. Saunders Company, London. 1119 pp. illus. £37.50. ISBN 0 7 16 1378 9.

With Dr Victor Chernick as a new associate editor and twenty-two new contributors, the third edition of Edwin L. Kendig's *Disorders of the respiratory tract in children* has become a still better textbook in paediatric pneumology than the former editions. All chapters have been rewritten or been revised and brought up to date. The seven new chapters dealing with important paediatric disorders have made this edition a very comprehensive book covering almost all paediatric diseases of the lungs. Most illustrations including roentgenograms are excellent and all chapters are followed by a lot of up-to-date references. Except some short chapters about disorders in adenoids and tonsils, this edition is limited to disorders in the lower respiratory tract. In spite of that, it has become a large book containing sixty-eight chapters and almost 1100 pages. It should be able to give answers to almost every clinical question about pulmonary diseases raised by physicians dealing with respiratory disorders in children.

Hans Ahlström

J. V. Simone (Guest editor) *Acute leukaemia. In Clinics in Haematology*. June 1978. 80 pp. illus. tabl. W. B. Saunders Company Ltd. \$16.00.

This book deals in particular with the therapeutic aspects of acute leukaemia. Clinical features, cytology and infectious complications are not covered in this issue of *Clinics in haematology*.

J. V. Simone has an excellent foreword with short comments on the topic's new since the 1977 edition on acute leukaemia. It is also interesting to note his remarks on editorial selection bias.

Five of the ten chapters are of particular interest to paediatricians. Stephen L. George points out the problems of design and evaluation of leukaemia trials. A. Mauer presents the principles of treatment of acute leukaemia in children and the current protocol studies from St. Jude Children's Hospital. The heterogeneity of the high risk group is stressed. The important discovery of abnormal computerized tomography of the brains of children receiving CNS-prophylaxis is mentioned and proposals for alternative CNS-prophylaxis not including irradiation are briefly commented on. All cases of AML off therapy at St. Jude Children's Hospital have subsequently relapsed.

Saunders and Donald Thomas from the Seattle group present an excellent survey and discussion of bone marrow transplantation, a procedure characterized by Simone as an experimental treatment method. Bone marrow transplantation has so far been most disappointing in leukaemias in Europe.

CNS and gonadal involvement are dealt with by Aur and Hustu in a chapter on extramedullary leukaemia (sanctuaries). The well known first 7 studies from St. Jude Children's Hospital (196-1971) are presented particularly with respect to their preventive CNS therapy. The frequency of CNS relapse was 8/45 (17.8%) in cases receiving cranial irradiation + methotrexate in study VII and the median duration of haematological remission 24 months (13 months for craniospinal group).

The increasing incidence of testicular involvement is also mentioned (28 to 97% at autopsy). Prophylactic irradiation of the testes is not used at St. Jude Children's Hospital and only one testis is irradiated in cases of unilateral leukaemic involvement.

Peter Johan Moe

for the acid dye eosin the eosinophil leukocyte has constantly puzzled its investigators. After reading the book by Beevon and Biss it is still far from clear what eosinophils do. The authors summarize not only in a purely descriptive but also in a highly critical way the existing knowledge of the basic nature of the eosinophil, its participation in phenomena of immunity, hormonal influences as well as extensive clinical observations of relevance to the subject.

At least the kinetics of the eosinophil seem well established today. It is likely that a key to the mysteries of what eosinophils do may lie in the specific functions of their granular proteins recently attracting increasing attention. The suggestion that the eosinophil has a homeostatic role in the immediate hypersensitivity reaction is of course highly likely, once in the inflammatory site it may interact with histophils and mast cells by several mechanisms. Otherwise a key factor in the genesis of eosinophilia appears to be processing of foreign material and parasites by cells in tissues. Interestingly T lymphocytes seem necessary participants in the subsequently accelerated marrow production of eosinophils. T lymphocytes may produce colony stimulating factors specifically for eosinophils. Also a low molecular weight eosinophilopoietin candidate was recently detected in serum of eosinophil depleted animals. The characteristic effects of corticosteroids are most likely due to reversible sequestration of the cells within the vascular compartment or the spleen. The benefit of the eosinophil is to be expected but is in the case of the neutrophil it may produce harmful effects too. Indisputable evidence indicates that prolonged high blood levels of eosinophils can cause a specific form of heart disease with endocardial fibrosis.

The monograph by Beevon and Biss on the eosinophil provides a most useful book for laboratory investigators and physicians. It gives a good theoretical understanding, and also shows our present lack of knowledge of the function of this cell.

Inge Olsson

P. I. Morselli (ed.) *Drug disposition during development*. 490 pp. illus. John Wiley & Sons Ltd, Sussex 1978. £28.20 \$50.00. ISBN 0 89335 006 0.

It was not possible until some 10–15 years ago to study in detail the kinetics of drugs in children. The recent progress is a result of the development of sensitive analytic techniques e.g. mass spectrometry, high pressure liquid chromatography etc. Most of the knowledge about drug disposition in children of various ages derives from the intense research during the last decade. The book by Paolo Morselli is an excellent and comprehensive monograph which brings together the current knowledge of drug disposition in the newborn, the infant and the child. Several authors, primarily from the Mario Negri Institute in Milan where important investigations in this research area have been carried out, contribute to the fifteen chapters of the monograph. The book starts with a rather detailed chapter on basic concepts of pharmacokinetics. This may serve as a useful repetition for the reader. The next few chapters concern some general aspects of clinical pharmacology, namely: Drug Absorp-

tion, Drug Plasma Protein Binding and Distribution.

Development of Drug Metabolizing Enzymes and Kidney Development, Drug Elimination Mechanisms with particular emphasis on the paediatric aspects. The other chapters describe the disposition of drugs belonging to certain groups: antineoplastic agents, antibiotics, chemotherapeutics, anticoagulants, antiinflammatory drugs, antiepileptics, hypnotics, cardiovascular agents and psychotropic drugs. All chapters are well written and seem to give a complete review of the current knowledge but would have gained a lot if the kinetic data had been tabulated to a larger extent. Also there is no comparative evaluation of the drugs in each group. Nevertheless, the book can be used with ease as an encyclopedia. It should serve as a very useful reference for investigators and paediatricians with special interests in pharmacokinetics and therapeutics for clinical pharmacologists, general practitioners and other medical people interested in the topic. Except for brief statements about the therapeutic indications of each drug, the book does not provide any therapeutic guidelines in different diseases, and was not intended to do so either. In such a case more information about drug effects and side-effects in infants and children would have been required.

Anders Rine

Maureen Oswin *Children living in long stay hospitals*. Spastics International Medical Publications (SIMPI). William Heinemann Medical Books Ltd, London.

This book is not a clinical description of paediatric long stay patients, but deals with the situation of 223 seriously mentally retarded children from a psychological-social educational point of view. The children live in special care wards in eight English hospitals for retarded children. All the children have multiple handicaps, most of them have no spoken language or ability to move. You find a high percentage of extra handicaps, as for example blindness and deafness. Judging from the descriptions the children must be seriously retarded.

According to the cover of the book the author is a teacher and has worked among retarded children since 1959. Already when seeing the title of the book you notice the interest and humane attitude of the author towards severely handicapped children. Children are children and live the same needs whether the diagnosis is psychiatric or a physical one. Apart from special needs linked to the handicap, children in different handicap groups have common natural basic needs. Here the author is consistent and the children are simply called mentally handicapped. The book has eight chapters which among other things describe the children, their families and special resources (physiotherapy, aids, speech therapy and some others). The need of medical consultants (paediatric and orthopaedic care) is also dealt with and the doctor is accused of not visiting the wards often enough and of not giving the child an adequate treatment. The book is full of illustrative examples.

The school situation of the children is dealt with in a special chapter. Compared to the situation in Sweden, English special schools have less resources. In spite of

this they are well known for creativity and good results. In one part of the book the situation of the nurses is described.

There are two appendices and the first one 'Scenes in Ward 7' could very well be a first independent chapter. Here Maureen Oswin describes every day situations from the different wards in a dramatic and touching way. Here you find things that the evening press could easily distort. Maureen Oswin's description is objective and although the contents of the book as a whole is critical, every chapter ends with constructive recommendations.

There is a list of recommended further reading. It is good but publications of James and Joyce Robertson are missing.

The book is good reading for everybody who has to do with mentally handicapped children (medical consultants, psychologists, teachers, nurses, administrators etc). The book can be highly recommended and should be available in every hospital library.

Peter Jørgensen

E. L. Kendig Jr & V. Chernick (eds) *Disorders of the respiratory tract in children* 3rd ed. W. B. Saunders Company, London, 1985, pp. illus. £3.90 ISBN 0 716-1178

With Dr Victor Chernick as a new associate editor and twenty-two new contributors, the third edition of Edwin L. Kendig's *Disorders of the respiratory tract in children* has become a still better textbook in paediatric pneumology than the former editions. All chapters have been rewritten or been revised and brought up to date. The seven new chapters dealing with important paediatric disorders have made this edition a very comprehensive book covering almost all paediatric diseases of the lungs. Most illustrations, including roentgenograms, are excellent and all chapters are followed by a lot of up-to-date references. Except some short chapters about disorders in adenoids and tonsils, this edition is limited to disorders in the lower respiratory tract. In spite of that, it has become a large book containing sixty-eight chapters and almost 1100 pages. It should be able to give answers to almost every clinical question about pulmonary diseases raised by physicians dealing with respiratory disorders in children.

Hans Ahlström

J. V. Simone (Guest editor) *Acute leukaemia*. In *Clinics in Haematology* June 1978, 60 pp, illus, tabl. W. B. Saunders Company Ltd, \$16.90.

This book deals in particular with the therapeutic aspects of acute leukaemia. Clinical features, cytology and infectious complications are not covered in this issue of *Clinics in Haematology*.

J. V. Simone has an excellent foreword with short comments on the topics new since the 1977 edition on acute leukaemia. It is also interesting to note his remarks on editorial selection bias.

Five of the ten chapters are of particular interest to paediatricians. Stephen L. George points out the problems of design and evaluation of leukaemia trials. A. Mauer presents the principles of treatment of acute leukaemia in children and the current protocol studies from St. Jude Children's Hospital. The heterogeneity of the high risk group is stressed. The important discovery of abnormal computerized tomography of the brains of children receiving CNS prophylaxis is mentioned and proposals for alternative CNS prophylaxis, not including irradiation, are briefly commented on. All cases of AML off therapy at St. Jude Children's Hospital have subsequently relapsed.

Saunders and Donald Thomas from the Seattle group present an excellent survey and discussion of bone marrow transplantation, a procedure characterized by Simone as an experimental treatment method. Bone marrow transplantation has so far been most disappointing in leukaemias in Europe.

CNS and gonadal involvement are dealt with by Aur and Hustu in a chapter on extramedullary leukaemia (sanctuaries). The well-known first 7 studies from St. Jude Children's Hospital (196-1971) are presented particularly with respect to their preventive CNS therapy. The frequency of CNS relapse was 8/45 (17.8%) in cases receiving cranial irradiation + methotrexate; in study VII and the median duration of haematological remission 74 months (73 months for craniospinal group).

The increasing incidence of testicular involvement is also mentioned (8 to 92% at autopsy). Prophylactic irradiation of the testes is not used at St. Jude Children's Hospital and only one testis is irradiated in cases of unilateral leukaemic involvement.

Peter Johan Moe

ANNOUNCEMENT

INTERNATIONAL WORKSHOP ON THE AT-RISK INFANT

An international workshop on the at-risk infant will be held in Tel Aviv, Israel, July 25-27, 30-31, 1979. The following topics will be discussed: Prospective parents, pregnancy and perinatal period, infancy and early childhood standards for socio-ecological community health care facilities. For further information write to: The secretariat, P.O. Box 16271, Tel Aviv, Israel.

DEVELOPMENTAL PAEDIATRICS¹

M. MANCIAUX

In the sixties some authors in the States have described the syndrome of the dissatisfied paediatrician resulting from too much work without great intellectual and human interest.

More recently the French paediatrician P. Royer, chairman of the International Paediatric Association, elaborated on what he called the syndrome of the anxious paediatrician. There are many reasons for this anxiety:

- the zero growth of the population resulting in too few children
- the better health status of the children group as a whole
- the increase in number and improvement in quality of health structures at various levels
- the general practitioners wanting more and more to take care of children
- the multiplication of paediatric subspecialties and of the non medical professions dealing with children

What is left to paediatricians?

However, in most developed countries:

- perinatal care is still insufficient
- one does not know much on infant and child morbidity
- there is a gap of surveillance and care between one year of age and school entrance
- there are still shortcomings and delays in the diagnosis, assessment and care of various handicapping conditions and chronic diseases
- attention and care for adolescents are badly lacking

These lacks and shortcomings plead for a more competent approach and interest by paediatricians.

However, most of these problems are psychosocial by nature. Is not the key point that most paediatricians are poorly, if at all, prepared to this stimulating approach which is the privileged field of social or better psychosocial paediatrics?

Social paediatrics has undergone a tremendous development in many countries in the last 20 years, but this does not reflect in the training of health and health related personnel. Moreover, in some countries it has become a kind of paediatric subspecialty which is a pity since all paediatrics should be preventive and social. In other countries it has fragmented into many topics: immunizations—public health related to childhood—child abuse and neglect—adolescent medicine—and so on, losing the totality and the continuity of the children's development and welfare.

That is the reason why I do prefer now to use the concept and approach of our British colleagues: Developmental paediatrics, enlarging it to encompass also the physical growth and the future of the child which is after all the main aim and outcome of growth and development.

In this concept, developmental paediatrics deals with:

- physical growth and maturation
- psychosocial development
- screening for health and disease
- prevention in childhood of aging and being later affected by degenerative diseases in old age

—
Curt Gyllensward lecture given October 27, 1978, Stockholm

If this breakdown is useful for analysis it could be misleading as to the global view of paediatric problems. We know how artificial it is to separate physical and psychosocial growth. To speak only about nutrition which could be seen as a problem purely related to physical growth, we are now aware of the ill effects on psychological development and school achievements, of too poor a diet during early life. On the other hand we know the so called psychosocial dwarfism caused by the lack of stimulation of the child by his parents or caretakers. So the reality of life is complex! However for making it easier we shall stick to our analytical approach starting with somatic development.

In the field of *physical growth* developmental paediatrics should first include methods of collecting relevant and readily obtainable somatic growth data. Among the problems to be clarified are

- the kinds of equipment to be used by the individual physician or paramedical workers
- which parameters are meaningful and usable
- the measuring techniques to be selected
- what kinds of graphs, curves, charts should serve as standards
- how often and at which ages should measurements be made (head, chest, arm)

Second, more widespread and better use of these data is necessary for evaluation of the individual status of children, e.g. the progress of a child born to parents who are either very tall or small can be interpreted correctly and differentiated from growth abnormalities due to inherited or acquired pathology, e.g. a more suitable selection of sports, physical exercises, may be provided for individual children after study of their biotype and their physical potentialities. Moreover it could be of interest to correlate more systematically the problems of poor school achievements with biologic maturation status, assessed for example by skeletal age measurement.

Third, applying this methodology to a large number of children using a well designed cross sectional protocol can result in the collection in a short period of time of valuable information on growth and development. This information should be used on a public health basis for secular comparisons between generations and for contemporaneous comparisons of children in different socio-economic groups and contexts.

Furthermore, growth data and charts derived from these data can be used for assessing the health of individuals and groups, whether in private practice or in health centres and clinics. The chart recently prepared by WHO for use in countries where no national references are available is a useful and appropriate tool. Unfortunately developmental charts appear not to be used extensively by the paediatricians or public health workers either in the developing or developed world. Developmental paediatrics should lead to a more accurate monitoring of children's growth and development both on an individual and a community basis. It is especially important to control carefully the physical as well as the psychological growth and development of chronically ill children, of children suffering from various kinds of handicaps etc. in order to help them to achieve as far as possible their genetic potential of development that might have been affected by the disease and sometimes by the treatment.

In the industrialized world *psychological development* is an area of child health which occupies or should occupy the attention of paediatricians to an increasing extent.

Diagnosis and evaluation of psychosocial development is and probably will continue to be difficult and will require the close cooperation of medical and non medical disciplines. These must include obstetrics, paediatrics, nutrition sciences, psychology, social work. It is a real problem to fulfil the needs in this field when working with a large number of children in health centres. In addition, measuring mental and emotional maturation

levels is much more difficult than measuring physical growth. The interpretation of psychosocial development may be hazardous and complicated.

In passing one should recall the abuses to which the intellectual quotient has been subjected and the damage which has resulted for individuals, groups and communities.

Attempts to evaluate emotional and psychosocial development of children from the early age are the basis of developmental paediatrics. Much effort has been made to set up standards for such an assessment and various scales have been prepared by specialists. Using material from these workers for assessing at key periods the level of psychosocial development of children needs to be intensively taught and practiced. The emphasis is now rightly put on the assessment and if necessary the reinforcement of the early mother/child relationship. However other periods of life also deserve increasing attention.

Throughout childhood and adolescence the main problems facing paediatricians in their daily practice pertain to the psychosocial field. It is this area in which paediatric skills should be sharpened so that parents, teachers, psychologists can be guided when they have to deal with diagnosis and management of learning disabilities, one of the commonest reasons for seeking paediatric help in the school age period.

Later on during preadolescence parents are often at a complete loss as to the handling of many difficult or impossible children both at home and at school as indicated by the widespread but so far ineffective PINS programme in the States (Persons In Need of Supervision).

Because of inadequate or totally lacking solutions to these preadolescent problems there is a great risk that these children will become maladjusted adolescents. In fact as clearly shown by data from all over the world delinquency is nowadays a problem of increas-

ing concern and no solutions have been found so far which can be extensively applied.

Drugs continue to spread among youth in most parts of the world. In the States where the phenomenon appeared 20 years ago alcohol is the commonest teen age drug. In a recent New York State schools survey between

- 60 and 90% of adolescents have been found using alcohol
- 40 and 60% of adolescents have been found using tobacco
- 6 and 40% of adolescents have been found using marijuana
- 5 and 15% of adolescents have been found using LSD
- 1 and 3% of adolescents have been found using heroin

The shifting from soft to hard drugs has been proved by several surveys. For instance the Columbia University Research conducted in some schools of New York on a representative sample of adolescents from 14 to 18 years old has shown that

- ~ 30% of youngsters who do not use any drug start alcohol and tobacco in the following 6 months
- ~ 27% using alcohol and tobacco shift to marijuana
- ~ 26% using marijuana try hard drugs after 6 months

Drug abuse in pregnancy has serious possible consequences for the passively exposed foetus since many of the preparations pass the placenta readily. Due to the high rate of pregnancies in teen agers the effects of drug usage by youth on foetal health and growth and on labour have serious implications well studied in countries like Sweden. It may be noted that already in 1971 more than 50% of deaths due to child abuse occurred in the States in families in which both parents were narcotic addicts.

Screening is the third chapter of developmental paediatrics deriving quite naturally

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Third, applying this methodology to a large number of children using a well designed cross sectional protocol can result in the collection in a short period of time of valuable information on growth and development. This information should be used on a public health basis for secular comparisons between generations and for contemporaneous comparisons of children in different socio-economic groups and contexts.

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Screening ■ the third chapter of developmental paediatrics deriving quite naturally

from the observation of physical and psychosocial growth and development

To elaborate on screening would take too long! Let us simply consider some theoretical points related to screening in its principle and practice

According to WHO screening is the presumptive identification of unrecognized disease or defects by the application of tests, examinations or procedures which can be applied rapidly. One could also add safely and at low cost at least for mass screening. In many cases selective screening is more valuable and cost/benefit acceptable. Based on epidemiological studies it aims at identifying causative factors and determining at risk/high risk groups through sound and feasible indicators, factors and indicators on which a screening procedure can be built.

Screening was first developed for disease. The criteria to be fulfilled are as follows. The disease is

- serious
- possible to diagnose
- treatable either medically or by rehabilitation and the prognosis is improved through early treatment
- there is an adequate screening period
- the disease is relatively prevalent
- the screening procedure is not harmful
- facilities are available for screening evaluation and treatment
- the procedure is of reasonable cost and
- acceptable to the population

(Frankenburg 1976)

Selection and use of screening tests and procedure must consider

- prevalence and natural history of the disease
 - target population
 - availability and cost of screening
 - definitive evaluation of positive patients
- (M. Gutgesell 1978)

The future of screening lies in a better fulfilment of criteria (in terms of cost/benefit cost/efficiency) in screening for handicapping

conditions. The full process of screening, assessment and rehabilitation should be established. Finally the screening procedure must be integrated into the full range of health services.

However the foreseeable future of screening will be to screen not only for disease but also for health and we should encourage the use of development screening tests by primary care paediatricians.

There again the practice is far from being satisfactory. For example a survey conducted in the USA has shown that paediatricians use development screening tests infrequently and usually only after evidence of development delay has been established by other criteria. We are far from the concept of screening for development and health as elaborated by Mary Sheridan and Ronnie McKeith in the early sixties!

Pediatrics is by nature preventive: since each single cure or cure intervention in childhood is aimed at safeguarding or restoring the health of the child not only for the present but also for a better future.

Moreover with the new knowledge concerning the possibilities of prevention of certain adult diseases based on biochemical and physiologic data derived from cross sectional studies, pediatrics becomes not only preventive but really *prospectiva*. This is the most fascinating aspect of developmental pediatrics based on scientific research as well as on carefully planned epidemiological surveys.

In 1974 a WHO consultative group reviewed the then current research related to the early possible childhood antecedents of atherosclerosis. It stressed the need for continued studies in this field and listed a number of problems which require elucidation.

Some risk factors were outlined which might suggest a risk pattern. Screening for these should be done in early childhood and should consist of data on family history, biochemical findings, blood pressure, weight, physical activity, cigarette smoking etc.

Half of the 8 or 10 risk factors related to coronary heart disease may be induced in childhood most of them are closely allied with the way of living especially with nutritional habits. However their degree of significance their persistence through adolescence and adulthood their combination in a risk pattern their potential dangers have not as yet been fully documented. This means that it is probably too soon to develop a body of preventive guidelines.

Many studies are under way some of them under WHO auspices which may help clarify this important public health problem. Meanwhile one can only attempt to prevent or treat especially in families at risk such harmful factors as overnutrition obesity hypertension cigarette smoking etc.

Closing a British symposium entitled 'Prevention of coronary heart disease starts in

childhood' O Wolff turned to the educative role of the paediatrician towards colleagues in the health and teaching fields who are in the best position to influence children and their families in making changes in eating and other habits. Paediatricians should seek to show these groups the importance of starting good habits in childhood. This is also developmental paediatrics!

ACKNOWLEDGEMENT

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ATTEMPT AT ENZYME REPLACEMENT IN GAUCHER DISEASE BY RENAL TRANSPLANTATION

C G GROTH H COLLSTE S DREBORG G HÅKANSSON G LUNDGREN and L SVENNERHOLM

From the Division of Transplantation Surgery Department of Surgery Karolinska Institutet Stockholm Huddinge Hospital Huddinge the Department of Neurochemistry University of Gothenburg and the Department of Paediatrics County Hospital Boden Sweden

ABSTRACT Groth C G Collste H Dreborg S Håkansson G Lundgren G and Svennerholm L (Division of Transplantation Surgery Department of Surgery Karolinska Institutet Stockholm Huddinge Hospital Huddinge the Department of Neurochemistry University of Gothenburg Gothenburg and the Department of Paediatrics County Hospital Boden Sweden) Attempt at enzyme replacement in Gaucher disease by renal transplantation *Acta Paediatr Scand* 68 475 1979—In Gaucher disease there is a deficiency of the lysosomal enzyme cerebrosidase as a result of which cerebroside (glucosylceramide) accumulates in various organs. In northern Sweden 22 patients with a juvenile form of this disease have been identified. In one such patient a girl of 10 years we have attempted enzyme replacement by renal transplantation. After this operation the hepatic glucocerebrosidase content fell significantly. In another child affected with Gaucher disease in whom splenectomy was performed for severe splenomegaly and hypersplenism there was a progressive increase in the level of this lipid. These findings suggest that enzyme replacement was achieved by transplantation of a normal organ.

KEY WORDS Gaucher disease enzyme replacement renal transplantation

In a patient suffering from an enzyme deficiency disease enzyme replacement could conceivably be achieved by transplantation of a normal organ. In Gaucher disease there is a deficiency of the lysosomal enzyme cerebrosidase as a result of which cerebroside (glucosylceramide) accumulates in various tissues and organs especially the spleen, liver and skeleton. In northern Sweden 22 patients with a distinct genetic form of the disease have been identified (5, 6). Although the time of onset and the course of the disease show considerable variations the majority of the patients suffer from a juvenile form of the disease. Early on massive deposits of cerebroside appear in the spleen. Splenectomy leads to accelerated cerebroside storage in the liver and skeleton and symptoms from the central nervous system (5, 6).

In one splenectomized child we have attempted enzyme replacement by renal trans-

plantation having first ascertained that renal tissue contained a high level of cerebrosidase. After the operation the hepatic cerebrosidase concentration was determined at intervals. The same studies were carried out in another Gaucher patient of the same age who underwent splenectomy for severe splenomegaly and hypersplenism but received no transplant.

MATERIAL AND METHODS

Enzyme and lipid assays

The cerebrosidase β -glucosidase activity in leukocytes and parenchymatous organs was measured with glucose [C]-cerebroside as a substrate (1). With this method the enzyme activity in leukocytes from normal subjects is $7.69 \pm 0.64 \mu\text{kat/kg}$ of protein in our laboratory.

The splenic and hepatic cerebrosidase concentrations were examined by thin layer chromatography and densitometric scanning of the charred cerebrosidase spot using cerebroside isolated from a Gaucher patient as a standard (2). In the liver and spleen from normal individuals the cerebrosidase content is less than $0.03 \mu\text{mol/g}$ of fresh

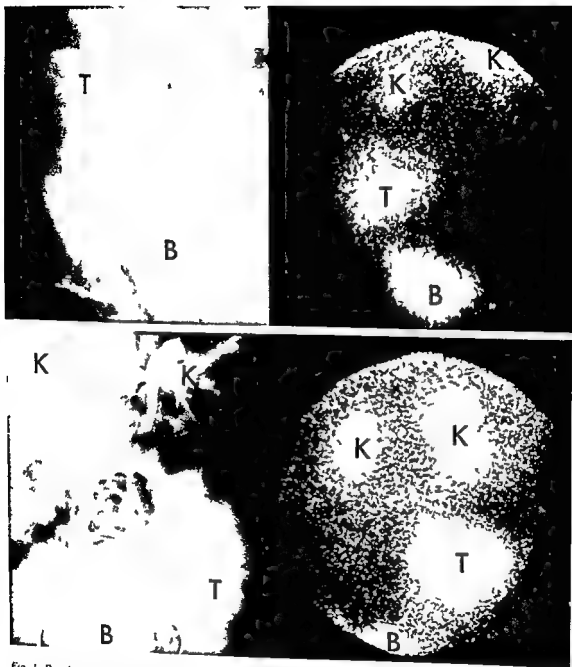


Fig. 1 Renal transplants in a patient with Gaucher disease. Second (upper) and third (lower) renal grafts visualized with intravenous pyelography and radio isotope scintigraphy.

and scintigraphy showed excellent graft function (Fig. 1). Three weeks later there was a deterioration of function and the prednisone dose was increased. Five weeks after the operation high fever and skeletal pains de-

veloped followed by dyspnoea and hypoxia. Antibiotics were given and the doses of the immunosuppressive drugs were reduced. Despite these measures diffuse pulmonary infiltration developed and death from hypoxia

tissue. The phospholipid content which was used as an indicator for the tissue state of hydration was estimated as inorganic phosphate after wet ashing (9).

In the 2 Gaucher patients liver specimens were obtained at intervals by open biopsy. The tissue was frozen immediately in liquid nitrogen and kept frozen until analysis. The frozen specimen was cut into thin sections weighing 10–20 mg each of which was submitted to analysis. Assays were performed on 5–20 pieces from each specimen. The assays on the specimens from the transplant recipient were performed on a single occasion after storage for up to 1 year. Although the specimens were wrapped in polyethylene film there had been a significant loss of water as evidenced by elevated and variable levels of liver phospholipid. In these specimens the best measure of changes in cerebroside content is provided by the cerebroside/phospholipid molar ratio. The assays on the biopsy specimens from the splenectomized patient were all performed without delay.

Normal tissue for measurement of enzyme activity

Cadaveric renal, hepatic and splenic tissue was harvested from 12 non-Gaucher subjects (aged 40–80 years). The specimens were obtained between 5 minutes and 12 hours after death and kept frozen until examined for cerebroside β glucosidase activity.

Patient undergoing renal transplantation

This was a 10 year-old girl with a known familial history of Gaucher disease. In infancy hepatomegaly, splenomegaly and pancytopenia had been diagnosed. At 6 years the large spleen (11 kg) had been removed. The cerebroside content was 44.9 $\mu\text{mol/kg}$ of fresh tissue (cerebroside/phospholipid ratio 2.96:1).

At 8–10 years skeletal destruction had developed which resulted in multiple spontaneous fractures and severe skeletal pain. There was moderate mental retardation. On admission to this Hospital the hepatomegaly was of an advanced stage and biopsy showed the presence of numerous Gaucher cells. The cerebroside β glucosidase activity in the leukocytes was 0.17–0.32 $\mu\text{kat/kg}$ of protein (6–12% of the normal activity).

Patient undergoing splenectomy

This was a 10 year-old boy with a known familial history of Gaucher disease. One of 4 siblings had died from the disease; the other 2 were not afflicted. When the boy was 2 years of age Gaucher cells were identified in a splenic biopsy specimen. There was both hepato- and splenomegaly. At 9 years skeletal destruction typical of the disease developed. The cerebroside β glucosidase activity in the leukocytes was 0.48 $\mu\text{kat/kg}$ (18% of the normal activity). At 10 years the spleen was so large as to greatly interfere with the child's mobility and there was thrombocytopenia with bleeding.

RESULTS

Enzyme activity in normal tissues

The mean cerebroside β glucosidase activities in the non-Gaucher renal, hepatic and splenic

tissue were found to be 9.9 ± 2.1 (S.D.) 4.1 ± 2.1 and 4.7 ± 2.1 $\mu\text{kat/kg}$ of protein respectively.

Patient undergoing renal transplantation

In August 1975 a cadaveric kidney was transplanted from a child of the same age as the recipient. There were 2 HLA A/B incompatibilities. The vascular anastomoses were to the right iliac vessels and the ureter was implanted into the bladder. The immunosuppressive agents were azathioprine and prednisone. As the patient's own kidneys functioned normally the condition of the graft could be appraised only by radiological examination and urine analysis.

Eight days after the operation there was evidence of acute rejection with fever, procteturia and an elevated amount of fibrinogen degradation products in the urine. The transplant was not visualized on scintigraphy or intravenous pyelography. Angiography revealed vascular abnormalities consistent with rejection.

On day 11 the transplant was removed and replaced by a cadaveric kidney from another child of the same age (3 HLA A/B incompatibilities). Microscopic examination of the first graft confirmed acute rejection.

The second transplant produced large volumes of urine immediately after revascularization. There was again an early acute rejection episode but this was reversed with prednisone. One month after transplantation the graft was excellently visualized by pyelography and scintigraphy (Fig. 1). During the next 7 months the clinical condition improved: the skeletal pains were less severe and mobility was increased.

Seven months after the operation fever developed. The graft was not visualized radiographically. On exploration it was found to be rejected and thrombosed and it was removed.

At the same operation a cadaveric kidney from an adult (2 HLA A/B incompatibilities) was anastomosed to the iliac vessels on the left side. Diuresis was immediate and pyelography

particularly by the liver cells where they would then degrade the stored cerebroside.

In the child undergoing splenectomy there was a progressive increase in the hepatic glucocerebroside content after the operation. This finding indicates that the spleen is a major reservoir for the cerebroside that the patient is unable to degrade when the spleen is removed; there will be a rapid increase in the liver's cerebroside content. Increasing deposits in other organs will probably also occur. Previously studied patients have displayed increasing skeletal involvement and mental retardation in the years after splenectomy (6). This suggests that any attempt at enzyme replacement therapy in Gaucher disease should be made at the time of splenectomy or soon afterwards.

Organ transplantation clearly holds promise as a means of enzyme replacement. The greatest impediment to lasting success is the destruction of the transplant by rejection.

REFERENCES

- 1 Brady RO, Kanfer JN & Shapiro D. The metabolism of glucocerebrosides. I. Purification and properties of a glucocerebroside-cleaving enzyme from spleen tissue. *J Biol Chem* 240: 39, 1965.
- Brady RO, Pentchev PG, Gal AE, Hibbert SR & Dekaban AS. Replacement therapy for in-

- herited enzyme deficiency. Use of purified glucocerebrosidase in Gaucher's disease. *N Engl J Med* 291: 889, 1974.
- 3 Desnick SJ, Desnick RJ, Brady RO, Pentchev PG, Simmons RL, Najarian JS, Swaiman K, Sharp HL & Krivit W. Renal transplantation in type II Gaucher's disease. In B Bergsma (ed.) *Enzyme therapy in genetic disease. Birth Defects Orig Art Series* 9: 107, 1973.
- 4 Groth CG, Hagenfeldt L, Dreborg S, Lofstrom B, Öckerman P, A Samuelsson E, Svennerholm L, Werner B & Westberg K. Splenic transplantation in a case of Gaucher's disease. *Lancet* i: 1760, 1971.
- 5 Herrlin K, M & Hillborg P, O. Neurological signs of a juvenile form of Gaucher's disease. *Acta Paediatr Scand* 51: 137, 1967.
- 6 Hillborg P, O & Svennerholm L. Blood level of cerebroside in Gaucher's disease. *Acta Paediatr Scand* 49: 707, 1960.
- 7 Neufeldt EF, Sando GN, Garvin AJ & Rome LH. The transport of lysosomal enzymes. *J Supramol Struct* 6: 95, 1977.
- 8 Schlesinger P, Rodman JS, Frey M, Lang M & Stahl P. Clearance of lysosomal hydrolases following intravenous infusion. The role of liver in the clearance of β glucuronidase and *N*-acetyl β -D-glucosaminidase. *Arch Biochem Biophys* 177: 606, 1976.
- 9 Svennerholm L & Vanier MT. The distribution of lipids in the human fetal and infant brain. *Brain Res* 47: 457, 1977.

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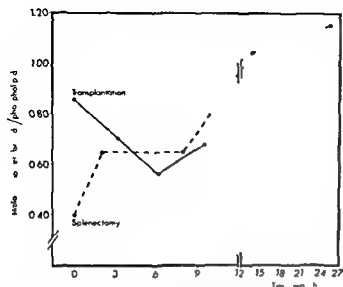


Fig 2 The hepatic cerebroside content in 2 patients with Gaucher disease: one undergoing renal transplantation for enzyme replacement; the other splenectomy because of severe splenomegaly. * denotes a significant change since the previous reading.

ensued 2 months after the third transplantation. At autopsy cytomegalovirus was isolated from the lung tissue. The renal transplant showed histologic changes indicative of mild chronic rejection.

Over the 7 months during which the second renal graft was functioning the glucocerebroside level in the recipient's liver fell from 44.0 to 24.3 $\mu\text{mol/g}$ of tissue, and the cerebroside/phospholipid molar ratio fell from 0.86 to 0.57. In the post mortem hepatic tissue the lipid had increased to 30.3 and the ratio to 0.73 (Fig 2). The cerebroside β glucosidase activity in the removed first graft was within the normal range for renal tissue: $9.1 \mu\text{kat/kg}$.

Patient undergoing splenectomy

Splenectomy was performed in April 1976. The enormous spleen weighed 2.4 kg. The cerebroside content was 33.2 $\mu\text{mol/g}$ net weight of tissue (cerebroside/phospholipid ratio 1.85). After the operation there was an improvement in the patient's mobility and the platelet count was normalized. Up to the time of this report 2 years after splenectomy the child's general condition has remained satisfactory but he has had at least one episode of

bone necrosis with infarction of the femoral neck.

Since splenectomy the hepatic glucocerebroside level has risen from 12.9 μmol to 34.8 and the cerebroside/phospholipid ratio from 0.40 to 1.15 (Fig 2).

DISCUSSION

We have previously attempted a splenic transplantation in a patient with juvenile Gaucher disease (4). During the 7 weeks that the graft was functioning there was indirect evidence of a beneficial metabolic effect, with a reduction in the serum level of acid phosphatases. An attempt at renal transplantation to a child with infantile Gaucher disease by Desnick et al was followed by a temporary clinical improvement (3).

It was formerly supposed that the cerebroside β glucosidase activity is particularly high in splenic tissue. Our knowledge of the activity of the enzyme in various organs has however been incomplete. Our own data led us to attempt to supplement the enzyme by renal transplantation.

The reduction in the cerebroside content of the liver in the patient carrying a renal transplant suggests that enzyme replacement was accomplished. The reason for the metabolic and clinical deterioration observed during the last few months is unclear. One possibility is that the infection from which the patient was suffering provoked an exacerbation of the metabolic disease.

Neufeld and co-workers have recently proposed that hydrolytic enzymes are introduced into lysosomes only after secretion and receptor-mediated capture; these mechanisms apparently operate in cultured fibroblasts (7). Lysosomal enzymes injected intravenously into rats are rapidly cleared from the circulation—mainly into the liver and spleen (8). It is tempting to speculate that the transplant will secrete lysosomal enzymes and that these would be taken up by various cells and par-

THE PROTECTIVE EFFECT OF ACUTE PHASE REACTANTS IN NEONATAL SEPSIS

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ABSTRACT Philip A G M (Department of Pediatrics University of Vermont USA) The protective effect of acute phase reactants in neonatal seps is *Acta Paediatr Scand* 68 481 1979.—Phase reactants were evaluated prospectively in babies suspected of having sepsis. Among 318 babies there were 27 proven and 10 very probable cases of neonatal sepsis. Of the proven cases 14 survived and 8 died. The survivors had a positive latex C reactive protein (CRP) in 11 cases and an alpha₁-acid glycoprotein (AGP) level greater than 0.5 g/l in 1 case. Among those who died one had a positive latex CRP and none had AGP >0.5 g/l. These findings were supported by positive CRP and elevated AGP in almost all very probable cases all of whom survived. These data in newborn infants support the hypothesis that acute phase reactants have a functional role in combating infection.

KEY WORDS Newborn sepsis acute phase reactants

In the presence of inflammation or infection the concentration of a number of serum proteins increases as the acute phase response. The evaluation of these acute phase reactants in the newborn period has been primarily directed at the diagnosis of infection. Acute phase reactants which have been evaluated in neonatal sepsis include C reactive protein (8), haptoglobin (9), α_1 acid glycoprotein (orosomucoid) (3, 10), fibrinogen and other proteins which influence the erythrocyte sedimentation rate (1, 7).

Little attention has been paid to the function of these acute phase proteins. C reactive protein has been described as a phagocytosis promoting factor (4). Recent experimental evidence indicates that in adults both C reactive protein (5) and α_1 acid glycoprotein (2) may play an important role in modifying lymphocyte responsiveness.

This report provides evidence in newborn infants which supports the hypothesis that acute phase reactants may be important in helping to combat infection.

MATERIALS AND METHODS

Acute phase reactants were evaluated in a prospective study of neonatal sepsis during the first week of life (6). Serum was obtained from 318 babies who were being investigated with a "sepsis work up" for either risk factors (e.g. prolonged rupture of membranes, smelly amniotic fluid, maternal fever, etc.) or clinical manifestations of neonatal sepsis (e.g. lethargy, abdominal distension, temperature instability, etc.).

There were 22 babies with proven bacterial sepsis who provide the main focus of this report. Serial diagnostic tests for sepsis were used including C reactive protein (CRP) and α_1 acid glycoprotein (AGP).

C reactive protein was measured with a latex reagent method which provides a positive reaction with levels equal to or greater than 8 mg/l.

Alpha acid glycoprotein (orosomucoid) was measured with an immunodiffusion method (1).

Low birth weight (LBW) is defined as less than 3500 g.

RESULTS

Of the 22 babies with proven bacterial sepsis 14 babies survived (7 LBW) and eight babies

Reagents kindly supplied by Behring Diagnostics
Somerville New Jersey

DISCUSSION

Evaluation of babies with proven bacterial sepsis showed that elevated levels of two proteins considered to be acute phase reactants were strongly correlated with mortality. Those newborn infants who had elevated levels of C reactive protein and α_1 acid glycoprotein when sepsis was first suspected survived. Those with low levels of CRP and AGP usually died. These findings strongly support the hypothesis that both CRP and AGP play an important role in the ability to combat infection.

Although there appears to be a strong influence of birth weight upon survival or death from neonatal sepsis there were several notable exceptions. It seems more likely that the very premature (preterm) newborn infant has difficulty with or is sometimes incapable of mounting an appropriate response to bacterial infection. When a baby is able to respond appropriately by producing (increasing) acute phase reactants there appears to be a protective effect.

The findings in the cases of proven bacterial sepsis support the hypothesis of a protective effect of acute phase reactants. Further support is provided by the elevated levels of CRP and AGP found in the cases of very probable infection all of whom survived. Elevated levels are infrequently seen when there is no evidence of infection. The values for CRP indicate that a positive response is not determined entirely by birth weight. Less than 10% of babies weighing more than 2500 g had a positive test and several very LBW babies were able to produce an increase in CRP. The mean level for AGP in heavier babies was not dissimilar to the total group.

The function of acute phase reactants has been speculative until recently. Evidence in vitro has indicated the importance of CRP and AGP in modulating lymphocytes (2, 5). CRP increased in vitro phagocytosis of *D. pneumoniae*.

Staph aureus, *E. coli* and *Klebsiella aerogenes* (4). The evidence presented here strongly suggests that in newborn infants acute phase reactants also have a protective effect against neonatal sepsis.

REFERENCES

- 1 Adler S N & Denton R L. The erythrocyte sedimentation rate in the newborn period. *J Pediatr* 86: 947 1975.
- 2 Chiu K M, Mortensen R F, Osmand A P & Gewurz H. Interactions of alpha acid glycoprotein with the immune system. I. Purification and effects upon lymphocyte responsiveness. *Immunology* 32: 997 1977.
- 3 Gotoh H, Ishikawa N, Shion T, Hatton Y, Nomura H & Ogawa J. Diagnostic significance of serum orosomucoid level in bacterial infections during neonatal period. *Acta Paediatr Scand* 62: 69 1973.
- 4 Kindmark C-O. Stimulating effect of C reactive protein on phagocytosis of various species of pathogenic bacteria. *Clin Exp Immunol* 8: 941 1971.
- 5 Mortensen R F & Gewurz H. Effects of C reactive protein on the lymphoid system. II. Inhibition of mixed lymphocyte reactivity and generation of cytotoxic lymphocytes. *J Immunol* 116: 1744 1976.
- 6 Philip A G S & Hewitt J R. Early diagnosis of neonatal sepsis. (Submitted for publication.)
- 7 Reher J P, Toubas P L & Minkowski A. Elevated fibrinogen in the diagnosis of bacterial maternal-fetal infection. In L Stern, B Friis-Hansen and P Kildeberg (eds). *Intensive care in the newborn*. Masson Publishing Co. New York 1976: 51.
- 8 Sabel K G & Hanson L A. The clinical usefulness of C reactive protein (CRP) determinations in bacterial meningitis and septicemia in infancy. *Acta Paediatr Scand* 63: 381 1974.
- 9 Salimi T T. Haptoglobin levels in the plasma of newborn infants with special reference to infections. *Acta Paed et Scand Suppl* 241 1973.
- 10 Sann L, Krawiecki N, Bourgeois J & Hermier M. Etude de l'orosomucoïde chez le nouveau-né infecté dans le diagnostic des infections bactériennes. *Arch Fr Pediatr* 33: 961 1976.

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Table 1 Outcome in babies with neonatal sepsis related to the presence or absence of a positive latex C reactive protein test

Latex CRP	Survived	Died	Mean birth weight (g)
-ve	3	7	1 789
+ve	11	1	2 423

died (7 LBW). The babies were classified according to whether they lived or died and with respect to C reactive protein (Table 1) and α_1 acid glycoprotein (Table 2). It is clear that when either of these two phase reactants was present in significant amount the likelihood of survival was very good. When low levels of phase reactant were present death from infection was likely.

The mean birth weight of the survivors was 2 517 g versus 1 466 g for those who died. However, it is pertinent to point out that one infant with birth weight 1 180 g had a positive CRP and AGP of 1.44 g/l and survived and another infant with birth weight 2 665 g had a negative CRP and AGP of 0.15 g/l and died of infection. It is also noteworthy that all of 10 infants with

Table 2 Outcome in babies with neonatal sepsis related to the level of α_1 acid glycoprotein (orosomucoid)

α_1 AGP (g/l)	Survived	Died	Mean birth weight (g)
<0.5	2	8	1 780
>0.5	12	0	2 431

clinical features strongly suggestive of sepsis but without a positive blood culture survived. Nine had AGP greater than 0.5 g/l and eight had a positive CRP (Table 3).

Evidence that birth weight may not be the major determinant is provided by the following facts. Babies with weights over 2 500 g without evidence of infection ($n=89$) had a mean level of AGP of 0.324 g/l compared to a mean level of 0.265 g/l for the total group without evidence of infection ($n=281$). There were 33 babies in this latter group who had AGP levels greater than 0.5 g/l. Of these 12 babies were LBW. CRP was positive in 19 babies without evidence of infection. Eleven of the 19 babies were low birth weight. The smallest baby with a positive test weighed 1 020 g.

Table 3 Findings in 10 babies who had strong presumptive evidence of infection but lacked a positive blood culture

S W U = sepsis work up G B S = Group B Beta hemolytic streptococcus

Identity no	Age at S W U	Birth weight (g)	Sex	CRP	α_1 AGP (g/l)	Factors suggesting infection
1/16	2 d	3 544	M	+ve	0.64	Pneumonia
1/41	8 h	2 041	M	-ve	0.84	W B C = 2 300/mm ³ pneumonia maternal G B S
1/104	12 h	2 840	M	+ve	0.39	Lethargy tracheal aspirate—G B S
1/112	2 d	4 111	M	+ve	0.66	Fever pustule grew staph aureus coag positive
1/166	4 d	2 280	M	-ve	0.67	Poor feeding shock W B C = 3 200/mm ³
2/40	2 d	2 060	F	+ve	0.58	Pneumonia tracheal aspirate—G B S
2/46	4 h	2 980	F	+ve	0.93	Apnea shock double dose antibiotics I V before transfer and before blood culture
2/99	6 h	3 232	F	+ve	0.91	Fever maternal G B S
7/114	4 h	4 000	M	+ve	0.96	Maternal fever and antibiotics tracheal aspirate—E coli antibiotics I V before transfer and before blood culture
2/120	3 d	2 395	F	+ve	>2.0	Pneumonia fever

LISTERIOSIS DURING PREGNANCY AND NEONATAL PERIOD IN SWEDEN 1958-1974

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ABSTRACT Larsson S Cronberg S and Winblad S (Departments of Infectious Diseases and Bacteriology University of Lund General Hospital Malmö Sweden) Listeriosis during pregnancy and neonatal period in Sweden 1958-1974 *Acta Paediatr Scand* ■ 485 1979 — In 1958-1974 altogether 46 cases of bacteriologically verified infection of *Listeria monocytogenes* were diagnosed during pregnancy and the neonatal period. Eight pregnancies resulted in abortion and three in stillbirth. Thirty seven children were born alive. 17 of whom died, all but one within a few days. These children were divided into three groups according to their age at the onset of illness: 22 cases with Early disease (≤ 2 days), four cases with Intermediate disease (3-5 days) and eight cases with Late disease (≥ 6 days). Three children were apparently healthy. Septicemia or Granulomatosis infantiseptica dominated in Early disease and claimed as many as 13 deaths. In Late disease all the children had meningo-encephalitis and only one of them died. The symptoms were typical of purulent meningitis. The group of Intermediate disease consisted of overlapping cases of the other two groups. Ampicillin alone or combined with gentamicin seemed to be the drug of choice in the therapy of neonatal listeriosis. Of the surviving children two were seriously damaged and two had moderate injuries. Fifteen children are apparently healthy. In cases where pregnancy terminated in abortions stillborns or children with Early disease the mothers often showed signs of infection. The mothers of the children with Late disease were apparently healthy. These children were infected from other sources, some of them nosocomially. If listeriosis is diagnosed during pregnancy the women should be treated with ampicillin.

KEY WORDS *Listeria monocytogenes*, pregnancy, neonatal period, abortion, stillborn, septicemia, meningo-encephalitis, infection in utero, nosocomial.

Pregnant women and newborns are predisposed to infection with *Listeria monocytogenes* (Lm) and listeriosis during pregnancy and the neonatal period is a common manifestation of the disease (3, 5, 24). The reason why the pregnant woman is susceptible is obscure but as a rule she is not seriously affected. When the foetus or the newborn is infected listeriosis often causes a serious infection with a high mortality. The first case of human infection diagnosed in Sweden was a boy with Granulomatosis infantiseptica reported in 1958 (19). This prompted Winblad (19) to register all bacteriologically verified cases of listeriosis in Sweden. The National Health Service made listeriosis notifiable as

from 1960. Part of this material—64 cases of listeriosis in children more than 27 days old and adults—has been published separately (17). The observations made in 46 pregnant women and their neonates are reported below.

MATERIAL AND METHODS

Patients. All cases of listeriosis diagnosed in pregnant women and newborns up to 7 days of age registered in 1958-1974 were included. The diagnosis had been verified by isolation of the microorganism in 20 cases from the women, in 47 cases from the foetuses/neonates and in 15 cases from both of them. Lm was isolated from the urethra, cervical and vaginal secretion, placenta, amniotic fluid, membranes, blood or faeces from the women and from cerebrospinal fluid (CSF), blood, throat, nose, eye, pustules and from various organs obtained at necropsy of the foetuses/neonates.

Table 3 Neonatal listeriosis in Sweden 1958-1974 34 cases

	Early disease (≤ 7 days)	Intermediate disease (3-5 days)	Late disease (≥ 6 days)
Septicæmia	19/37	7/4	0/8
Meningitis	3/7	7/4	8/8
Mortality	13/37	4/4	1/8
Maternal fever	9/7	1/4	1/8
Discoloration of amniotic fluid	16/31	1/3	1/5
L.m. pos. culture in mother	10/14	1/7	0/4
L.m. pos. culture in child	22/37	4/4	8/8
Birth weight g (median)	7 440		3 460
Gestational age weeks (median)	37		38
Pos. serology for listeria	14/16	1/3	0/3
Complicated parturition	8/7	0/4	1/8

O-agal. titer $> 1/40$ and/or CF titer $> 1/10$

(Table 3) The median birth weight was significantly higher in this group than in the group of Early disease (Rank sum test $p < 0.05$). The mortality was low.

Most of the children with septicæmia were in a bad condition already at birth. Nineteen had signs of respiratory distress and circulatory failure. Seventeen children had neurological symptoms, mostly flaccidity or increased tone with twitching. Seizures occurred in eight of these children, seven of whom died. All four children with petechiae died. Vomiting and/or diarrhoea occurred in four children and enlargement of the liver in six. Nine out of 17 children studied got fever and eight children, including five with a birth weight of less than 2500 g, had a low or normal temperature.

In the cases of meningo-encephalitis the symptoms were typical of purulent meningitis with fever, irritability, whimpering and some

times loss of appetite. A tense fontanelle was noted in six cases. Six children had seizures which was fatal in one. Four children had respiratory symptoms, five had vomiting and/or diarrhoea and five children had enlargement of the liver. All children, except one weighing less than 2500 g, had fever.

In the primary CSF the number of polymorphonuclear as well as of mononuclear cells was increased (Table 4). Polymorphonuclear cells dominated in eight children and mononuclear cells in five. Protein in the CSF was substantially increased (Table 4) and the glucose concentration was decreased in only three out of five cases studied.

Antimicrobial treatment. Since these patients had been treated in different hospitals there was no uniformity of the chemotherapeutic treatment for which reason caution should be exercised in the evaluation of the

Table 4 Findings in primary CSF in meningo-encephalitis in neonatal listeriosis in Sweden 1958-1974 13 cases

	No. of cases	Median value	Range
Total number of cells per l	13	3.630×10^6	$198-6.600 \times 10^6$
Polymorphonuclear per l	11	1.963×10^6	$23-3.400 \times 10^6$
Mononuclear per l	11	1.000×10^6	$763-3.767 \times 10^6$
Protein/g per l	10	~ 0	$1.9-10.74$
Polymorphonuclears in relation to mononuclears	13	Poly $>$ Mono 8	Mono $>$ Poly 5

Number of cases



Fig. 1. Annual incidence and outcome of neonatal listeriosis in Sweden 1958-1974. 46 women (2 pairs of twins). ■ = abortions □ = livebirths (survivors) ▨ = stillborns ▩ = livebirths (succumbed later)

Clinical information was obtained from a study of all patients' records and was sometimes supplemented by direct information from the patients and their relatives or from members of the hospital staff.

Part of this material has been published earlier in case reports (2, 6, 8, 9, 10, 12, 14, 19, 21, 22, 26, 27). Seasonal variation, geographic distribution, animal contacts, serotypes and other epidemiological aspects are reported elsewhere (15). The pathological changes found at necropsy are published separately (18).

Bacteriology. The technique used for bacteriological culture and diagnosis of *Listeria monocytogenes* has been described elsewhere (15).

Serology. Antibodies against *L. m.* were assayed according to Winblad (15, 28).

RESULTS

After the discovery of the first case in 1958 there were rather many cases in 1959 and 1960. Since then the frequency has been rather even and low (Fig. 1).

Clinical manifestation

Eight pregnancies ended in abortion and three in stillborn foetuses (Table 1). Thirty-seven children were born alive and 21 of these had septicemia. A name we prefer to the earlier designation 'Granulomatosis infantiseptica'.

Table 1. *Listeriosis during pregnancy in Sweden 1958-1974*

Clinical diagnosis: 46 women (two pairs of twins)	
Abortion	8
Stillborn	3
Children born alive	
Granulomatosis infantiseptica	21 (15 fatal)
Meningo-encephalitis	13 (1 fatal)
No listeria infection	3 (1 fatal)

Table 2. *Abortions and stillborns in listeriosis in Sweden 1958-1974: 11 cases*

Abortion	8/11
Stillborn	3/11
Maternal fever	5/11
Discoloration of amniotic fluid	7/11
<i>L. m.</i> pos. culture in mother	8/9
<i>L. m.</i> pos. culture in foetus	8/8
Serology positive	6/8

O aggl. titer > 1/40 and/or CF titer > 1/10

which proved fatal in 15. Thirteen children had meningo-encephalitis which was fatal in one. Three of the infected women delivered children with apparently no clinical or bacteriological signs of listeriosis. One of these children was born in the 34th week of pregnancy and soon died because of its immaturity.

Clinical course in the children

Infection in utero resulted in abortion in eight and stillborns in three cases (Table 2). *L. m.* was isolated from various organs.

The infected children born alive were divided into Monnet's (20) three groups according to their age at the onset of neonatal listeriosis: 22 cases with Early disease (≤ 2 days), four cases with Intermediate disease (3-5 days) and eight cases with Late disease (≥ 6 days).

Early disease (22 cases) Septicemia dominated this group (Table 3). Two children had meningo-encephalitis but this was probably only part of a septicemia. *L. m.* was isolated from various organs. Median values of birth weight and gestational age indicated that the pregnancies had not gone on to full term. The mortality was high (13 cases).

Intermediate disease (4 cases) Two of the patients had septicemia and two had meningo-encephalitis (Table 3). *L. m.* was isolated from various organs. In the two fatal septicemic cases birth weight was lower than that of the two who survived from meningo-encephalitis.

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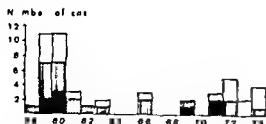


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Table 5 Comparison between distribution of maximal antibody titers in two materials of registered blood donors and three materials of listeriosis

Category	Total	O aggl pos ($>1:40$) (%)	CF pos ($>1:10$) (%)	O aggl pos CF pos (%)
Blood donors 1971-1975	53	8	4	16
Blood donors 1977	100	9	4	2
Women with listeriosis during pregnancy	77	78	63	59
Mothers of children with late type of listeriosis	4	0	0	0
Adults and juveniles with listerial meningitis and septicemia	35	49	40	6

much less common. One woman was delivered by Caesarian section.

The placenta. Eighteen women had pathological changes in the placenta. Another 26 placentae were examined macroscopically only and found to be normal. All seven placentae analysed in cases of abortion and stillbirth had pathological changes and all but one had signs of placentitis. In the group of

Early disease four women had pathological changes with infarctions, clots and/or calcifications and another three women showed signs of placentitis. One woman in the group of Late disease had pathological changes with calcifications and duplex placenta.

Serotype of *L. m.* The distribution of serotypes 1 and 4b was fairly uniform (Table 6).

A few strains were diagnosed as serotype 2 and 3. Epidemiological aspects of the serotypes are discussed in an earlier paper (15).

Antimicrobial treatment. Since the women were treated in different hospitals the chemotherapy of listeriosis was not uniform. Caution must therefore be exercised in the evaluation of the effect of the treatment. Two women were treated during pregnancy. One woman with SLE developed septicemia during pregnancy and was treated with benzyl penicillin 10 MU a day parenterally. She had an abortion after eight days of therapy. The other woman was treated with a sulphonamide because of symptoms of urinary tract infection. After four days blood culture yielded growth of *L. m.* and ampicillin parenterally was given.

Table 6 Distribution of serotypes in relation to clinical diagnosis in Sweden 1958-1974

Serotyping according to Winblad. Early disease consisted of 19 gran infantisept and 3 men encephalit. Intermediate disease consisted of 1 gran infantisept and 7 men encephalit. Late disease consisted of 8 men encephalit.

	Serotype					Total
	1	3	4b	Unknown		
Abortion	4	1	1	2	8	
Stillborn	1			2	3	
Early disease (≤ 4 days)	9	2	8	2	21	
Intermediate disease (4-5 days)	1		1	2	4	
Late disease (≥ 6 days)	5	1	2		8	
No illness			3		3	
	0	4	15	8	48*	

* Nine cases were serotyped by Seeliger, Würzburg, Germany.
* 46 women with two pairs of twins.

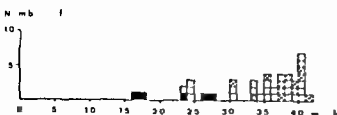


FIG. 2. Gestational age in listeriosis in Sweden 1959-1974. 41 neonates and foetuses: ■ = abortions, ▨ = still births, ▤ = live births with meningoencephalitis, ▥ = live births with septicemia, □ = live births with no listeria infection.

effect of chemotherapy. Sulphonamide combined with other drugs was successful in four cases. It failed in one case. Tetracycline combined with other drugs was successfully used in all five cases in the beginning of the period covered by the material. Ampicillin combined with gentamicin in four cases and ampicillin combined with other drugs in three cases was successful. In two cases this treatment failed. In 17 surviving cases the therapy was continued for a median period of 20 days (range 8-41 days).

Outcome. Most of the deaths in the group. Early disease occurred within two days. A girl with meningoencephalitis got hydrocephalus and died later (at 65 days) from seizures and respiratory arrest.

Twenty children survived the neonatal listeriosis. Two children got hydrocephalus and serious mental retardation, one child had a slight spastic diplegia and one developed convergent strabismus.

A mongoloid girl recovered from septicemia but died at three months from gastroenteritis. Fifteen children are apparently healthy with normal psychomotor development. The surviving children were followed up by pediatric specialists during a median period of 20 months. Fourteen children were followed up for at least one year.

Clinical course in the mothers

The median age of the mothers was 27 years (range 17-39 years). Twenty five of them had been pregnant before.

Forty three women were apparently

healthy. One woman had systemic lupus erythematosus (SLE). Another woman was treated during pregnancy because of thyrotoxicosis and one woman had rheumatoid arthritis. None of the women were treated with corticosteroids or cytostatics.

Laboratory findings. A few women (10 cases) tested showed moderate leukocytosis (median value 12.0×10^9 , range $4.3-28.4 \times 10^9$). All these women had growth of L.m. Of nine women studied, five had neutrophilic granulocytosis and none had monocytosis. The erythrocyte sedimentation rate (ESR) was raised (median 69 mm, range 20-113).

Gestational age. All the cases of listeriosis occurred after the 16th week of pregnancy (Fig. 2).

Serology. Serological analysis was done in 31 cases (Table 5). Listerial infection in pregnancy resulted in raised antibody titers significantly more often than after meningitis and septicemia in adults and juveniles ($p < 0.05$). Of 11 women studied within six days after delivery, ten had already developed positive titers.

Seventeen women had apparently had normal pregnancies and four out of nine studied had growth of L.m. in the genital tract. Another 17 women showed signs of infection and 11 out of 13 examined had L.m. in the genital tract. Usually the women had chills and fever a few days before delivery combined with muscle pain, headache and tiredness. Five women showed signs of urinary tract infection. After delivery they recovered and became afebrile, most of them rather soon without or in association with the beginning of antibiotic treatment. Other complications like bleeding, hypertension with edema and albuminuria and premature contractions occurred in another 12 women. In 21 the amniotic fluid was discolored.

The mothers of children with Early disease often showed signs of infection (Table 3). In eight of these women parturition was complicated. In the mothers of the children with Late disease signs of infection were

nancy terminated in foetal death. Unfortunately neither necropsy nor culture was done in these two cases.

DISCUSSION

The present investigation confirmed and extended earlier observations (20) that neonatal listeriosis occurs in two distinct forms which differ in time of onset, mode of transmission, clinical symptoms and prognosis.

In this study 22 infants fell ill within the first two days of life. Typically they had septicaemia with nonspecific signs of respiratory distress and circulatory failure that were evident already at birth (1, 3, 24). Seizures and petechiae indicated a bad prognosis. In these cases the infection must have been transmitted during intrauterine life. The amniotic fluid was nearly always discolored and the mothers had typical fever. Growth of *L. m.* in the genital tract and positive serology. Most of the infants were born prematurely and had low birth weights. They were in a bad condition and the mortality was high, most of them dying within two days.

In contrast, all eight infants with late disease had meningo-encephalitis. They were born at expected time and had normal birth weights. When they fell ill after five days of life, they developed the usual signs of purulent meningitis with fever. Mononuclears often dominated the cells in the primary CSF suggesting virus or other agents. The definite diagnosis, however, should be based on isolation of the microorganism (13, 24). Their mothers were healthy. The amniotic fluid was normal. In no case was *L. m.* cultured from the genital tract. Serology remained negative. Thus the disease was evidently transmitted from the environment. In other studies nosocomial outbreaks of late disease have been reported (15, 16). The prognosis was good and all but one survived.

In no case infection during delivery was suspected, but since culture of the genital tract was seldom performed in late disease, the material did not exclude this possibility.

Infants falling ill when 3-5 days old belonged to an intermediate group of overlapping cases. Two were clinically of early type and two were clinically of late type.

Stillborns and abortions showed signs of intrauterine infection and resembled early disease.

As in other manifestations of listeriosis (3, 15, 17, 24), serotypes 1 and 4b dominated and were not correlated with the type of infection or the severity of the disease.

An earlier calculated perinatal mortality of about 1% (14) was not confirmed. As reported elsewhere (15), our material showed that listeriosis was diagnosed annually in up to 1 per thousand of all stillborns and infants who died within the first week of life (perinatal mortality). Of the total neonatal mortality in Sweden, listeriosis was the cause of up to 3.7 per thousand of all fatal cases within the first 27 days of life. The results resemble those of Breuning & Fritzsche (4) who found a death rate of 0.154% due to listeriosis.

Among the surviving children, four had sequelae after the infection. Out of 48 pregnancies, 15 children are still living and apparently healthy. This indicates the severity of listeriosis. If the physician suspects listeriosis, antibiotic treatment should be started immediately.

The effect of chemotherapy in the children was difficult to evaluate. Ampicillin was effective in some cases and is to be regarded as the main drug in the treatment of listeriosis in newborns (25). In vitro studies suggest that combined ampicillin and gentamicin should be used (7). Erythromycin is an alternative. See Liger (25) recommended continuation of treatment for up to 21 days.

Co-existing disorders in the mothers were not common but did occur in a few women. The higher frequency of raised antibody titers in pregnant women may have been due to the fact that most of them were previously healthy while adult patients with other manifestations of listeriosis were often immunocompromised.

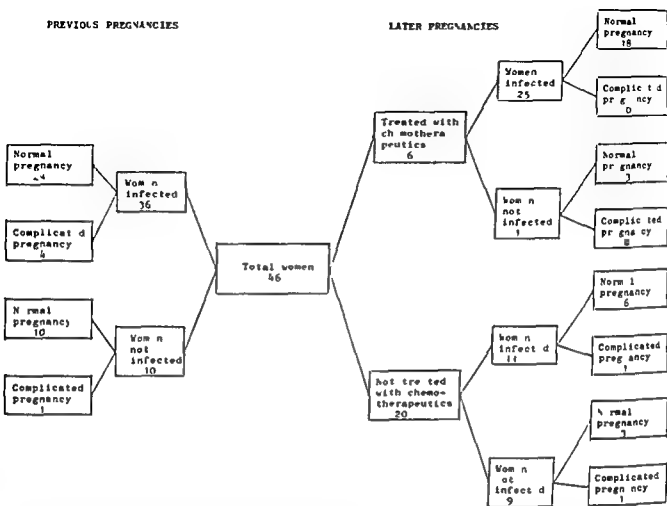


Fig. 3 Previous and later pregnancies in 46 women with listeriosis in Sweden 1948–1974. Women with septicaemia, abortion, stillbirth, or mothers of children with Early disease or early type of Intermediate disease were

classified as women infected. Mothers of children with Late disease or late type of Intermediate disease were classified as women not infected.

instead of sulphonamide. By then she had already become afebrile. Two days later she gave birth to a healthy boy.

In 20 women no chemotherapy of listeriosis was given after delivery. In another 24 women such treatment was given after delivery. Ampicillin (7 patients), penicillin (7 patients) and tetracycline (5 patients) were most commonly used alone or combined with other drugs. The duration of treatment ranged from four to 32 days (median 10 days).

Previous and later pregnancies. Before the pregnancy with listeriosis 34 pregnancies were normal and five were complicated, including four abortions and one case of vacuum extraction because of asphyxia (Fig. 3). One woman had two abortions. One of these

fetuses was examined at necropsy and showed no signs of infection. There was no statistical significant difference in the frequency of complicated pregnancies in women who were later apparently infected with *L. monocytogenes* in women who were not infected.

Twenty-six women treated with chemotherapy later had altogether 21 pregnancies without complications. In the group of 20 women not treated with chemotherapy there were later nine normal pregnancies and two complicated ones (Fig. 3). One woman got a stillborn. The death was caused by listeriosis. Later on she had an abortion. Another woman had a child with the Late disease from neonatal listeriosis, probably a case of nosocomial infection. A later preg-

nancy terminated in foetal death. Unfortunately neither necropsy nor culture was done in these two cases.

DISCUSSION

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There was no early abortion in our material and this is only rarely described (3, 24).

Two women were treated with antibiotics during pregnancy. In one of them treatment with benzyl penicillin could not prevent the infection of the foetus resulting in abortion. The woman, however, suffered from SLE with depressed cellular immunity which is most important for the resistance to listeriosis (5, 11). The other woman was treated with a sulphonamide and later with ampicillin and delivered a healthy boy. If listeriosis is diagnosed in a pregnant woman ampicillin should be used. Erythromycin is an alternative.

Many authors recommend antibiotic treatment of the mother when listeriosis is diagnosed after the delivery. The present experience did not indicate any advantage of such treatment (Fig. 3). Only two pregnancies in women who were not treated resulted in abortion and stillbirth respectively and in neither case was it proved that the fetal issue was due to listeriosis. However, Rappaport et al. (23) and other authors have reported cases of women with repeated abortions in their opinion caused by a persistent infection of the genital tract with *L. m.* Boysen Møller (3) was not convinced and stated that so far no definite case has been reported in which *L. m.* has been isolated twice in the same patient in two successive abortions or births. No prospective investigation of this question is on record. For the time being treatment of a mother with clinical or bacteriological signs of infection is recommended. Ampicillin is to be preferred. In patients allergic to penicillin tetracycline, erythromycin or cotrimoxazole are the alternatives. Seeliger (25) recommends oral treatment for 8–10 days.

REFERENCES

- 1 Becroft D M O, Farmer K, Seldon H J, Sowden R, Stewart J H, Vines A & Watlie D A. Epidemic listeriosis in the newborn. *Br Med J* III 747 1971.
- 2 Bergman P, Kjellander J, Thunell G & Zetterberg L. Listerios och graviditet. *Läkartidningen* 57 24 1960.
- 3 Boysen Møller J. Human listeriosis. Diagnostic epidemiological and clinical studies. *Acta Pathol Microbiol Scand (B)* Suppl 229 1972.
- 4 Breuninger M & Fritzsche F. Über die Häufigkeit der Listeriose bei Neugeborenen. *Geburtshilfe Frauenheilkd* 14 1113 1954.
- 5 Buchner L H & Schneidersson S S. Reviews. Clinical and laboratory aspects of *Listeria monocytogenes* infections. With a report of ten cases. *Am J Med* 45 904 1968.
- 6 Ekelund H, Laurell G, Melander S, Olding L & Vallqvist B. *Listeria* infection in the foetus and the newborn. *Acta Paediatr Scand* 51 698 1962.
- 7 Espize E P, Audouin A & Courtieu A L. Action in vitro of some antibiotic combinations on *Listeria monocytogenes*. *Proceedings of the 11th International Symposium on the Problems of Listeriosis* Varna Bulgaria 1977. In press.
- 8 Gullberg S, Jernelov H & Kahlmeter O. Tre fall av listeriemeningit. *Läkartidningen* 57 2117 1960.
- 9 Hagberg B, Olding L & Philipsson L. Two cases of listeria infection in the newborn. *Acta Paediatr Scand* 49 631 1959.
- 10 Hedberg K, Magnusson P H & Naglo O. Ett fall av listerios. *Opuscula Medica* 10 365 1959.
- 11 Ivarsson S, Lidin Jansson G & Svensson R. Listeric meningitis in the non-compromised host. *Infection* 5 204 1977.
- 12 Kayser F H & Malmström-Groth A G. Listerios—ytterligare fall hos spädbarn. *Läkartidningen* 57 797 1960.
- 13 Kromann Andersen C. Human listeriosis. En oversigt og fire tilfælde. *Ugeskr Laeger* 132 1310 1970.
- 14 Larsson J. Forekomsten av listeriainfektion i Sverige. *Läkartidningen* 57 37 1960.
- 15 Larsson S. Epidemiology of listeriosis in Sweden 1958–1974. Accepted for publication in *Scand J Infect Dis*.
- 16 Larsson S, Cederberg Å, Ivarsson S, Svanberg L & Cronberg S. *Listeria monocytogenes* causing hospital acquired enterocolitis and meningitis in newborn infants. *Br Med J* II 473 1978.
- 17 Larsson S, Cronberg S & Winblad S. Clinical aspects on 64 cases of juvenile and adult listeriosis in Sweden. *Acta Med Scand* 204 503 1978.
- 18 Larsson S & Linell F. Correlations between clinical and post mortem findings in listeriosis. Accepted for publication in *Scand J Infect Dis*.
- 19 Linell F, Schiller B & Winblad S. Listeriainfektion hos människor. *Nord Med* 61 181 1959.
- 20 Monnet P. Listeriosis in a neonatal unit. In M Woodbine (ed.) *Problems of listeriosis*. Leicester University Press, Sutton Bonington 1975. p 188.
- 21 Olding L & Philipsson L. Two cases of listeriosis in the newborn associated with placental infection. *Acta Pathol Microbiol Scand* 48 24 1960.
- 22 Palmén K & Sommerschild H. Perinatal listeria meningit. *Läkartidningen* 61 1781 1964.
- 23 Rappaport F M, Rabinovitz R T & Krochik N. Genital listeriosis as a cause of repeated abortion. *Lancet* I 1273 1960.
- 24 Seeliger H P R. Listeriosis. S Karger A G. Basel 1961.

- 5 Seeliger H P R & Mathers H Review Treatment of human listeriosis *Ger Med Mon* 14 566 1969
- 6 Stormby K Wallengren O & Weiber A Två listenosfall i nyföddhetsperioden med troligt nosokomalt smittosamband *Läkartidningen* 57 712 1960
- 7 Söderling L Listeriosis i Jundaregionen 1971-1973 *Läkartidningen* 71 7878 1974
- 8 Winblad S Studies in antibodies in human listeriosis I Antibodies in cases of bacteriologically verified human listeriosis *Acta Pathol Microbiol Scand* 58 13 1963

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Table 1 Occurrence of precipitins against *E. coli*, *B. fragilis* and *P. aeruginosa* in sera from normal persons

	Number of normal persons	Prevalence of persons with		
		0 precipitins	1 precipitin	2 precipitins
<i>E. coli</i>	44	32 (73%)	9 (20%)	3 (7%)
<i>B. fragilis</i>	44	37 (84%)	7 (16%)	
<i>P. aeruginosa</i>	154	145 (94%)	9 (6%)	

From (10)

study. These patients are examined every month as outpatients at the Pediatric Clinic TG. The diagnostic criteria for CF have been reported previously (5). The monthly examination included bacteriological examination of sputum or secretion obtained by endotracheal aspiration (6). Anaerobic culture of these respiratory secretions was not attempted since this is meaningless if percutaneous transtracheal aspiration is not employed in view of the abundance of anaerobes present in the normal flora of the upper respiratory tract (3). However, microscopic examination of the secretions provided no evidence of anaerobes as being important pathogens in the respiratory tract of our CF patients. Sera were obtained by venipuncture and stored at -30°C with 15 mM NaN₃/l added. The sera from CF patients who had previously harboured *E. coli* in the respiratory tract were collected on an average of 6 months (range 0-36 months) after the latest isolation of these bacteria. Twenty one of the CF patients have succumbed but serum from these patients was available from a large collection of specimens stored as described. These sera were collected on an average ½ months before death (range 1 day-18 months).

Control persons

Sera from 154 normal persons covering all age groups comprise the control group concerning *P. aeruginosa* precipitins (10) and sera from 44 normal persons (19 males, 25 females) the control group as regards precipitins against *E. coli* and *B. fragilis*. The mean age of these 44 persons was 20 years (range 1-59 years) and 30 of them were younger than 30 years.

Precipitating antibodies against *P. aeruginosa*, *E. coli* and *B. fragilis*

Crossed immunoelectrophoresis (microtechnique (7, 21, 22)) was employed to examine serum from the patients for precipitating antibodies against antigens obtained by sonication of the three bacterial species (5, 9). The *P. aeruginosa* antigen sample contains at least 64 different antigens (5, 11). Colloid concentration is 11.8 g/l (refractometry, human IgG as standard).

The *E. coli* (021:H27) antigen sample contains at least 42 antigens visualized as 42 immunoprecipitates with a corresponding rabbit antiserum in crossed immunoelectrophoresis, colloid concentration 14.8 g/l and 37 of the 42 antigens are common *E. coli* antigens (9).

The *B. fragilis* ss *thetaiotaomicron* (VPI 5) antigen sample contains at least 34 antigens visualized as 34 im-

munoprecipitates when run against a corresponding rabbit antiserum in crossed immunoelectrophoresis, colloid concentration 5.8 g/l and 19 of the 34 antigens are common antigens of other *B. fragilis* subspecies (9). Only one of the *P. aeruginosa* antigens cross reacts with *B. fragilis* ss *thetaiotaomicron* and five of the *P. aeruginosa* antigens and one *B. fragilis* ss *thetaiotaomicron* antigen cross react with *E. coli* (9).

By means of crossed immunoelectrophoresis the antibody response against *P. aeruginosa* is quantified by the number of precipitins (= the number of immunoprecipitates) which is correlated with the titres of the precipitins (11) and the same approach is used to quantify the antibody response against *E. coli* and *B. fragilis*.

Statistical methods

The χ^2 Square test and the Mann-Whitney test (7, 17) were used. Level of significance 5% (double tailed test).

RESULTS

A significantly higher prevalence of precipitins against *E. coli* was found in CF sera (76%) as compared with the normal sera (27%) ($p < 0.0005$) and 38% of the CF sera contained ≥ 3 *E. coli* precipitins (Fig. 1 and Table 1). The mean number of precipitins in positive

Table 2 Occurrence of precipitins against *E. coli* in sera from cystic fibrosis patients who have harboured and those who have not harboured *E. coli* in the lungs

	CF without <i>E. coli</i>	CF with <i>E. coli</i> ^a	Total
0-2 precipitins	55 (67%)	27 (33%)	82
≥ 3 precipitins	37 (73%)	14 (27%)	51
Total	92 (69%)	41 (31%)	133
		n.s.	

^a Average observation time 4.9 years.

^b Average observation time 3.3 years.

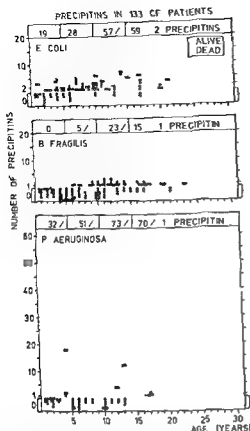


Fig. 1 Number of precipitins against *E. coli*, *B. fragilis* and *P. aeruginosa* in serum of 133 cystic fibrosis patients plotted against the age of the patients. ○ Patients still alive ● patients who subsequently succumbed. The highest number of precipitins against these bacteria revealed in sera from normal persons is indicated by the broken lines. As regards these lines the prevalences of increased numbers of precipitins against the bacteria in the different age groups are given at the top of each diagram (*E. coli* > 2 precipitins, *B. fragilis* > 1 precipitin, *P. aeruginosa* > 1 precipitin).

sera was 3 (range 1–12) and the prevalence of precipitins increased significantly with age ($p < 0.005$) (Fig. 1). The antibody response against *E. coli* was not correlated with previous colonization of the respiratory tract by these bacteria (Table 2) nor with the prognosis (Fig. 1).

A significantly higher prevalence of precipitins against *B. fragilis* was found in CF sera (38%) as compared with the normal sera ($p < 0.01$). The CF sera contained

≥ 2 *B. fragilis* precipitins (Fig. 1 Table 1). The mean number of precipitins in positive sera was 1.4 (range 1–4) and the prevalence of precipitins increased significantly with age ($p < 0.0005$) (Fig. 1). The antibody response against *B. fragilis* was not correlated with the prognosis (Fig. 1).

A significantly higher prevalence of precipitins against *P. aeruginosa* was found in CF sera (63%) as compared with normal sera ($p < 0.0005$) and 55% of the CF sera contained ≥ 2 *P. aeruginosa* precipitins (Fig. 1 Table 1). The mean number of precipitins in positive sera was 1.6 (range 1–60). The prevalence of precipitins increased significantly with age ($p < 0.0005$). The prevalence of ≥ 2 precipitins was significantly higher in the CF patients who had harboured these bacteria in the respiratory tract as compared with those who had never done so ($p < 0.0005$) (Table 3). The number of precipitins was significantly higher in these chronically infected patients (mean 22) as compared with the CF patients with only intermittent *P. aeruginosa* colonization (mean 2, $p < 0.0001$). The number of *P. aeruginosa* precipitins was significantly higher in CF patients who have subsequently succumbed (mean 27 precipitins) compared with the patients who are still alive (mean 12 precipitins, $p < 0.0005$) (Fig. 1).

The prevalence and number of precipitins in CF patients was significantly higher against *P. aeruginosa* than against *E. coli* and *B. fragilis* ($p < 0.001$) (Fig. 1). Tables 4 and 5 show that the presence of increased numbers of *E. coli* and *B. fragilis* precipitins was associated significantly with increased numbers of *P. aeruginosa* precipitins.

DISCUSSION

The antibody response against *P. aeruginosa* was much more pronounced than that against *E. coli* and *B. fragilis*. Besides being correlated with chronic *P. aeruginosa* lung infection and poor prognosis, the antibody response against *P. aeruginosa* has also been shown to be cor-

Table 3 Occurrence of precipitins against *P. aeruginosa* in relation to the intermittent or chronic presence of *P. aeruginosa* in the lungs of cystic fibrosis patients (6-11)

	CF without <i>P. aeruginosa</i>	CF with <i>P. aeruginosa</i> intermittently	CF with <i>P. aeruginosa</i> chronically	Total
0-1 precipitin	35 (58%)	25 (42%)		60
≥ 2 precipitins	1 (1.5%)	14 (19%)	58 (79.5%)	73
Total	36 (27%)	39 (29%)	58 (44%)	133

$p < 0.0005$

related with inflammation and tissue damage the presence of immune complexes in serum and especially in sputum and poor lung function (7-11, 14). In contrast the antibody response against *E. coli* and *B. fragilis* was not correlated with respiratory infections nor with the prognosis of the CF patients and infections outside the respiratory tract have not been observed in our patients (6-11). Accordingly the question is whether antibodies against *E. coli* and *B. fragilis* may be induced without the presence of infections caused by these bacteria.

Increased antibody response against these bacteria was found predominantly in CF patients with increased antibody response against *P. aeruginosa*. Several explanations for this interesting association may be offered (i) Cross reactive *P. aeruginosa* antigens may account for at least some of the antibody response against *E. coli* in some of the patients. This is less probable in view of the antibody response against *B. fragilis* which only cross

reacts with one of the *P. aeruginosa* antigens as compared with *E. coli* which cross reacts with five of the *P. aeruginosa* antigens (9). (ii) The presence of some *P. aeruginosa* antigens e.g. the lipopolysaccharide might induce an adjuvant effect on the antibody response to other antigens (13, 16). However as immunosuppressive effects of *P. aeruginosa* have also been reported (15) this explanation is doubtful. (iii) In accordance with the hypothesis of Willwork et al. (19) it is possible that the antibody response against *E. coli* and *B. fragilis* is induced by antigens from these species which have been absorbed from the gut.

A generalized increased absorption of antigens from mucosal surfaces due to a transient defect of secretory IgA seems less likely in view of the relatively low prevalence of antibodies against the abundant intestinal flora (*E. coli* 10^8 - 10^9 and *B. fragilis* 10^{11} /g dry weight of faeces (3)) as compared with the high prevalence of antibodies against the res

Table 4 Relationship between occurrence of precipitins against *P. aeruginosa* and *E. coli* in sera from cystic fibrosis patients

	0-2 <i>E. coli</i> precipitins	≥ 3 <i>E. coli</i> precipitins
0-1 <i>P. aeruginosa</i> precipitin	50 (37.5%)	10 (7.5%)
≥ 2 <i>P. aeruginosa</i> precipitins	32 (24%)	41 (31%)

$p < 0.0005$

Table 5 Relationship between occurrence of precipitins against *P. aeruginosa* and *B. fragilis* in sera from cystic fibrosis patients

	0-1 <i>B. fragilis</i> precipitin	≥ 2 <i>B. fragilis</i> precipitins
0-1 <i>P. aeruginosa</i> precipitin	60 (45%)	1 (1%)
≥ 2 <i>P. aeruginosa</i> precipitins	60 (45%)	12 (9%)

$p < 0.005$

piratory pathogen *P. aeruginosa* although the systemic antibody response against antigens absorbed from the intestinal tract to the venous blood is possibly less pronounced due to filtration by the liver (1).

However the malabsorption theory (19) may be modified to explain the results of the present work. Antigens from the gut flora which cross react with *P. aeruginosa* antigens must be present in the gut of all CF patients (9). Furthermore *P. aeruginosa* antigens must be present in the gut of the patients who harbour these bacteria in the respiratory tract due to sputum being swallowed. The presence of such antigens in the gut provides the possibility of immune reactions in the gut mucosa between these antigens in the gut and *P. aeruginosa* antibodies originating from serum in patients with chronic *P. aeruginosa* lung infection. Such immune reactions have been shown to increase absorption of other antigens through mucosal membranes *in vitro* (18) and evidence of immune complexes in biopsies from the gut of succumbed CF patients has actually been reported (12). A transient secretory IgA defect (20) would probably augment the possibility of such reactions.

In conclusion evidence has been presented which points to increased absorption of antigens from the gut of CF patients. It is suggested that the increased absorption is secondary to immune reactions in the intestinal mucosa.

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REFERENCES

1. Bjørneboe M, Frytz H & Ørskov F. Antibodies to intestinal microbes in serum of patients with cirrhosis of the liver. *Lancet* i 58 197.
2. Dem K (ed.) *Diagnostica Geigy Scientific Tables*. J. A. Geigy S. A. Basel 196. pp 36, 174.
3. Finegold S M. *Anaerobic Bacteria in Human Disease*. Academic Press New York 1977. pp 26, 41, 73.
4. Gibbons A, Allan J E, Holzel A & McFarlane H. Cell mediated immunity in patients with cystic fibrosis. *Br Med J* i 170 1976.
5. Høiby N & Axelsen N H. Identification and quantitation of precipitins against *Pseudomonas aeruginosa* in patients with cystic fibrosis by means of crossed immunoelectrophoresis with intermediate gel. *Acta Pathol Microbiol Scand (B)* 81 298 1973.
6. Høiby N. Epidemiological investigations of the respiratory tract bacteriology in patients with cystic fibrosis. *Acta Pathol Microbiol Scand (B)* 87 541 1974.
7. Høiby N. *Pseudomonas aeruginosa* infection in cystic fibrosis. Relationship between mucoid strains of *Pseudomonas aeruginosa* and the humoral immune response. *Acta Pathol Microbiol Scand (B)* 87 551 1974.
8. Høiby N & Wink A. Antibacterial precipitins and autoantibodies in serum of patients with cystic fibrosis. *Scand J Respir Dis* 56 38 1975.
9. Høiby N. Cross reactions between *Pseudomonas aeruginosa* and thirty six other bacterial species. *Scand J Immunol* 4 (Suppl 2) 187 1975.
10. Høiby N. Antibodies against *Pseudomonas aeruginosa* in serum from normal persons and patients colonized with mucoid or non mucoid *Pseudomonas aeruginosa*: results obtained by crossed immunoelectrophoresis. *Acta Pathol Microbiol Scand (C)* 85 147 1977.
11. Høiby N. *Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey. *Acta Pathol Microbiol Scand (C)* Suppl 26 pp 3-96 1977.
12. McFarlane H, Holzel A, Brechley P, Allan J, D. Wallwork J, C. Singer H E & Worslev B. Immune complexes in cystic fibrosis. *Br Med J* i 4 3 1975.
13. Sasaki M, Ito M & Homma J Y. Immunological studies on the original endotoxin protein (OEP) of *Pseudomonas aeruginosa*. Adjuvant effect of OEP *in vivo*. *Japan J Exp Med* 45 335 1975.
14. Schütz P O, Nielsen H, Høiby N, Gluckmann G & Svehag S E. Immune complexes in the sputum of patients with cystic fibrosis suffering from chronic *Pseudomonas aeruginosa* lung infection. *Acta Pathol Microbiol Scand (C)* 86 77 1978.
15. Schwab J H. Suppression of the immune response by microorganisms. *Bacteriol Rev* 39 171 1975.
16. Skidmore B J, Chiller J M, Morrison D C & Weigle W O. Immunologic properties of bacterial lipopolysaccharide (LPS): correlation between the mitogenic adjuvant and immunogenic activities. *J Immunol* 114 770 1975.
17. Therkelsen A J. *Medicinsk Statistik*. Akademisk Boghandel Århus 1968. pp 140 146.
18. Tolo K, Brandtzaeg P & Jansen J. Mucosal penetration of antigen in the presence or absence of serum derived antibody. *Immunol* 33 733 1977.

Table 3 Occurrence of precipitins against *P. aeruginosa* in relation to the intermittent or chronic presence of *P. aeruginosa* in the lungs of cystic fibrosis patients (6-11)

	CF without <i>P. aeruginosa</i>	CF with <i>P. aeruginosa</i> intermittently	CF with <i>P. aeruginosa</i> chronically	Total
0-1 precipitin	35 (58%)	25 (42%)		60
≥ 2 precipitins	1 (1.5%)	14 (19%)	58 (79.5%)	73
Total	36 (27%)	39 (29%)	58 (44%)	133

 $p < 0.0005$

related with inflammation and tissue damage the presence of immune complexes in serum and especially in sputum and poor lung function (7-11, 14). In contrast the antibody response against *E. coli* and *B. fragilis* was not correlated with respiratory infections nor with the prognosis of the CF patients and infections outside the respiratory tract have not been observed in our patients (6-11). Accordingly the question is whether antibodies against *E. coli* and *B. fragilis* may be induced without the presence of infections caused by these bacteria.

Increased antibody response against these bacteria was found predominantly in CF patients with increased antibody response against *P. aeruginosa*. Several explanations for this interesting association may be offered. (i) Cross reactive *P. aeruginosa* antigens may account for at least some of the antibody response against *E. coli* in some of the patients. This is less probable in view of the antibody response against *B. fragilis* which only cross

reacts with one of the *P. aeruginosa* antigens as compared with *E. coli* which cross reacts with five of the *P. aeruginosa* antigens (9). (ii) The presence of some *P. aeruginosa* antigens e.g. the lipopolysaccharide might induce an adjuvant effect on the antibody response to other antigens (13-16). However immunosuppressive effects of *P. aeruginosa* have also been reported (15); this explanation is doubtful. (iii) In accordance with the hypothesis of Wallwork et al. (19) it is possible that the antibody response against *E. coli* and *B. fragilis* is induced by antigens from these species which have been absorbed from the gut.

A generalized increased absorption of antigens from mucosal surfaces due to a transient defect of secretory IgA seems less likely in view of the relatively low prevalence of antibodies against the abundant intestinal flora (*E. coli* 10^8 - 10^9 and *B. fragilis* 10^{11} /g dry weight of faeces (3)) as compared with the high prevalence of antibodies against the res

Table 4 Relationship between occurrence of precipitins against *P. aeruginosa* and *E. coli* in sera from cystic fibrosis patients

	0-2 <i>E. coli</i> precipitins	≥ 3 <i>E. coli</i> precipitins
0-1 <i>P. aeruginosa</i> precipitin	50 (37.5%)	10 (7.5%)
≥ 2 <i>P. aeruginosa</i> precipitins	32 (24%)	41 (31%)

 $p < 0.0005$ Table 5 Relationship between occurrence of precipitins against *P. aeruginosa* and *B. fragilis* in sera from cystic fibrosis patients

	0-1 <i>B. fragilis</i> precipitin	≥ 2 <i>B. fragilis</i> precipitins
0-1 <i>P. aeruginosa</i> precipitin	60 (45%)	1 (1%)
≥ 2 <i>P. aeruginosa</i> precipitins	60 (45%)	12 (9%)

 $p < 0.005$

SUPPRESSED LYMPHOCYTE MITOGEN RESPONSIVENESS IN URINARY TRACT INFECTIONS OF CHILDREN AND ITS CORRELATION TO PYELONEPHRITIS

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ABSTRACT Janas M, Vesikari T, Hallström O and Anttila R (Departments of Paediatrics and Microbiology, Tampere Central Hospital and Institute of Clinical Sciences, University of Tampere, Tampere, Finland). Suppressed lymphocyte mitogen responsiveness in urinary tract infections of children and its correlation to pyelonephritis. *Acta Paediatr Scand* 68: 501, 1979. —Cell mediated immunity (CMI) was studied in a group of 48 children with urinary tract infections (UTI) using a whole blood micromethod for lymphocyte stimulation *in vitro*. The patients were subdivided into pyelonephritis group (27 cases) and lower urinary tract infection (LUTI) group (21 cases) on the basis of fever, erythrocyte sedimentation rate, C reactive protein and renal concentration capacity. At the acute stage of infection the lymphocyte responsiveness to leucoagglutinin (LA) and concanavalin A (Con A) was suppressed in both groups but the suppression was much greater in those with pyelonephritis. By 6 weeks after infection the lymphocyte responses were normal in most but not all cases. We conclude that an acute pyelonephritis is associated with marked suppression of CMI and that the latter can be used as an additional criterion for establishing the level of infection. Patients with UTI did not generally appear to have any primary defect of CMI but when suppression of CMI was present it seemed secondary to an ongoing infection.

KEY WORDS Cell mediated immunity, lymphocyte stimulation, urinary tract infections, pyelonephritis.

In the pathogenesis of urinary tract infections (UTI) in children bacterial interaction with uroepithelial cells and anatomical defects of the urinary tract are believed to play major roles (5, 14). Humoral immunity in terms of serum immunoglobulin levels and specific antibody response to bacterial antigens seems generally intact (5, 8).

Defective cell mediated immunity (CMI) could conceivably contribute to the pathogenesis of recurrent UTI either as a primary deficiency or having been temporarily suppressed by an external cause such as viral infection. UTI itself, particularly pyelonephritis, may also suppress CMI (9, 12). Both normal and impaired CMI have been reported in chronic and recurrent UTI in children but the various mechanisms have not been considered.

We report lymphocyte stimulation *in vitro*

as a measurement of CMI by the acute, early convalescent and late convalescent stages of UTI in children. Cases with pyelonephritis and lower urinary tract infection (LUTI) were analysed separately. Since the immunosuppression appeared to bear correlation to the level of infection the lymphocyte stimulation test was considered as an additional parameter in the level diagnosis of UTI (in addition to fever, C reactive protein (CRP), erythrocyte sedimentation rate (ESR) and renal concentration capacity).

MATERIALS AND METHODS

Patients

48 patients, 45 girls and 3 boys, presenting with acute UTI to the Department of Paediatrics, Tampere Central Hospital were studied. The diagnosis of UTI in most cases was based on significant bacterial growth in urine obtained by bladder aspiration. Alternatively, two clean voided

- 19 Wallwork J C, Brechley P, McCarthy J, Allen J D, Moss D, Ward A M, Holzel A, Williams R F & McFarlane H. Some aspects of immunity in patients with cystic fibrosis. *Clin Exp Immunol* 18: 303, 1974.
- 20 Wallwork J C & McFarlane H. The sIgA system and hypersensitivity in patients with cystic fibrosis. *Clin Allergy* 6: 349, 1976.
- 21 Weeke B. General remarks on principles, equipment, reagents and procedures. *Scand J Immunol* 7 (suppl. 1): 15, 1973.
- 22 Weeke B. Crossed immunoelectrophoresis. *Scand J Immunol* 2 (Suppl. 1): 47, 1973.

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Table 1 Level diagnosis of UTI by standard criteria in 48 patients. All patients in the pyelonephritis group had fever 38.5°C or more

Criteria	Positive/cases studied			
	Pyelonephritis (n=27)		LUTI (n=21)	
ESR ≥ 5 mm/h	21/21	100%	3/16	19%
CRP ≥ 10 μ g/ml	18/21	86%	0/16	0%
Urine osmolality <800 mOsm/l	0/21	95%	4/11	25%

Lower urinary tract infection

(Sigma). Each mitogen was used at 7 different concentrations. The concentration of LA ranged from 50 μ g/ml to 0.5 μ g/ml and that of Con A from 500 μ g to 5 μ g/ml. In addition for each test three cultures were set with no added mitogen.

All the results are expressed as stimulation ratios (s/r) obtained by dividing the counts in stimulated cultures (mean of three) by those in background cultures (mean of three). For statistical analysis of the results Student's *t* test was applied.

CRP determinations

CRP concentrations were measured by a single radial immunodiffusion method (10) using LC Partigen CRP kit of Behring Institut.

Renal concentrating capacity

The patients' ability to concentrate urine was usually determined on the second day of hospitalization. Two successive urine specimens were collected after 15 hours fasting. The osmolality of the urine was measured by a freezing point osmometer. The concentration capacity was considered reduced only if the osmolality of the urine in both samples was below 800 mOsm/l.

Diagnostic criteria for pyelonephritis

The criteria for level diagnosis of UTI proposed by Jodal et al. (6) were applied in a slightly modified form. A case of UTI was classified as pyelonephritis if 3 of the following 4 criteria were met: Fever 38.5°C or more, ESR ≥ 5 mm/hr or more, CRP ≥ 10 μ g/ml or more, and renal concentrating capacity <800 mOsm/l (see Results).

According to these criteria 27 cases were classified as pyelonephritis. Of the remaining 21 cases none was positive for more than one of the above criteria. Regardless of the degree of symptoms, all these cases were grouped as LUTI.

RESULTS

Normal dose response curve for LA and Con A

The best responsiveness of lymphocytes to LA was seen using the highest concentration of LA, 50 μ g/ml. A still higher concentration, 100 μ g/ml, did not improve the results but was not inhibitory either (data not shown). There was gradual decrease of stimulation with lower concentrations of LA and no stimulation was seen with 0.5 μ g/ml. The dose response curve of lymphocytes to Con A was similar except that the highest concentration, 500 μ g/ml, was found to be inhibitory (Fig. 1). The differences between the mitogens were probably related to the purity of reagents.

Maximal stimulation ratios with each mitogen at optimal concentrations were similar (Fig. 1). Individual variation in the control subjects was such that 2 standard deviations of the mean almost equalled maximal stimulation ratio.

Table 2 Lymphocyte responsiveness to leucoagglutinin in patients with pyelonephritis and LUTI at various stages of the infection

Days after admission	LA stimulation ratio compared to controls	Number of patients with decreased response			
		Pyelonephritis (n=27)		LUTI (n=21)	
0	Below -2.0 S.D.	15/27	56%	11/21	52%
	Below -1.0 S.D.	14/27	52%	15/21	71%
1-3	Below -0.5 S.D.	0/27	0%	0/21	0%
	Below -1.0 S.D.	14/27	52%	12/21	57%
4-5	Below -0.5 S.D.	2/27	7%	0/21	0%
	Below -1.0 S.D.	4/27	15%	7/21	33%

See text for explanation

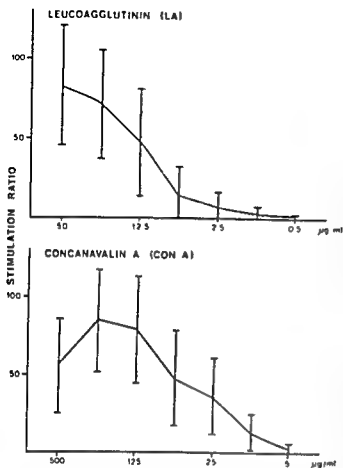


Fig. 1 Lymphocyte stimulation in whole blood cultures with different concentrations of leucoagglutinin and concanavalin A of 35 control children. The curves show mean stimulation ratios for each mitogen and the bars represent ± 1 standard deviation of the mean.

urine specimens yielding bacterial growth of the same strain over 100 000 per ml were required for diagnosis. Most patients were initially treated in the hospital but were discharged after the urine no longer yielded significant growth of bacteria. The therapeutic regimen

varied according to the clinical picture, level of diagnosis and bacteriological results.

In the pyelonephritis group the mean age was 5.0 years (range 2 months to 11 years 3 months) and in the LUT group 5.4 years (range 2 months to 11 years 7 months). In 27 cases there were no previous histories of UTI; the remaining 21 patients (9 in the pyelonephritis group and 12 in the LUT group) had had one or more previous episodes of UTI.

The first blood specimen for lymphocyte stimulation and other laboratory studies was collected prior to the start of antibiotic therapy. The second specimen was taken 14 days after the first; there was no more than 7 days variation in the collection of the specimens. A third specimen was taken 6 weeks after the first one.

The control group for the lymphocyte stimulation test consisted of 35 children with a mean age of 6.4 years (range 2 to 15 years). All were in good health, the majority coming to the hospital for elective surgery. The remainder were hospitalized for various investigations.

Lymphocyte stimulation test

The method was based on the whole blood culture technique with different concentrations of mitogens described by Park & Good (11) substituting gamma counting for liquid scintillation counting (4).

The cultures were established on U bottom Cooke Microtiter® plates (M24 ARTL). Into each well 0.075 ml of freshly collected heparinized whole blood and 0.1 ml of Eagle's MEM (Orion Diagnostica) containing the mitogen were added. The cultures were set in triplicate for each concentration of the mitogens. The incubation time was 72 hours at 37°C in an atmosphere containing 5% CO₂. Six hours before harvest 125 µCi of ³H-thymidine (Amersham) was added in 0.075 ml of MEM. The cells were collected using a multiple cell culture harvester (Skatron Flow Laboratories) on glass fibre discs which were dried and counted in a Wallac gamma counter.

Two mitogens were used: Leucoagglutinin (LA), a purified component of phytohemagglutinin (Pharmacia Fine Chemicals) and crude concanavalin A (Con A).

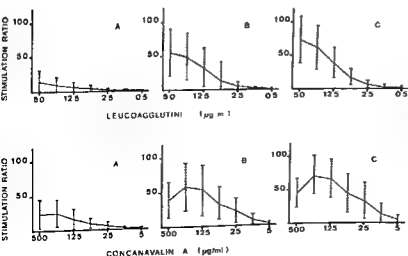


Fig. 2 Lymphocyte stimulation with leucoagglutinin (above) and concanavalin A (below) in 27 children with pyelonephritis. The curves show mean stimulation ratios and the bars ± 1 S.D. of the mean. The dotted area represents ± 1 S.D. of the control children. The lymphocyte stimulation was studied at the acute stage (A) at 2 weeks (B) and at 6 weeks (C) after infection.

experiments Miller et al noticed marked suppression of splenic T lymphocyte PHA responsiveness after experimental pyelonephritis. In their model little change was seen in peripheral blood lymphocyte function (9). Williams et al found a clear correlation between renal abscess formation and decrease in splenic lymphocyte response to Con A. In their study if artificial inoculation produced renal abscesses the lymphocyte stimulation indices were highly significantly reduced whereas in cases with kidney infection without abscesses there was only slight although significant suppression of lymphocyte responsiveness (12).

Our series of lymphocyte stimulation studies may represent a human counterpart for the immunosuppression observed in experimental pyelonephritis by Williams et al (12). One half of the patients with pyelonephritis showed almost total unresponsiveness of lymphocytes whereas in all of those with LUTI the immunosuppression was much less. The criteria used by us in the level diagnosis of UTI were in direct measures of systemic response to infection and did not necessarily reflect actual pathology in the kidneys. However if the observations from the animal model can be applied to human disease patients with seriously impaired CMI might also have abscess formation in the kidneys and the degree of acute stage immunosuppression in UTI could be an important indicator of the seriousness of the infection process at kidney level.

After treatment of the infection the lymphocyte responses returned to normal in most patients. There were however a few notable exceptions. These were in most cases associated with recurrent UTI, signs of chronic pyelonephritis or another infection elsewhere. Consequently lymphocyte stimulation studies might be of significance in the monitoring of recovery and in detecting chronic infection. It must be borne in mind however that suppression of lymphocyte mitogen responsiveness is not specific for UTI but may be associated with other viral or bacterial infection (7).

Since decreased lymphocyte responsiveness in the late convalescent stage in most cases was seen only together with an associated cause we believe that none of the patients had a primary defect of CMI. It is difficult however to assess the role of impaired CMI in the recurrence of UTI. A recurrent or chronic UTI may cause immunosuppression but CMI suppressed by other causes might precipitate recurrence.

The present whole blood method for lymphocyte stimulation appears rapid and simple enough to include in the laboratory work up of patients with UTI. Of the two mitogens used in the study LA is more suitable since it causes no agglutination of red blood cells. Testing with different concentrations of a mitogen may not be necessary. The suboptimal concentrations of LA and Con A were used in the study as it is known that a slight suppression of lymphocyte stimulation is more readily detected under such conditions (2, 11). This was really not necessary in the present work as a marked immunosuppression was seen in most patients. Furthermore the normal variation in the controls was of such a degree that a suspected slight decrease in lymphocyte responsiveness should not be given too much attention. Although the present limits of -1.0 S.D. and -2.0 S.D. of the mean were arbitrary measures of decreased CMI they may represent right orders of magnitude for probable and significant immunosuppression respectively.

The mechanism of impaired CMI in acute UTI remains unclear and was not elucidated to any greater degree in the present study. However it did not appear to be due to serum factors since purified lymphocytes from a few patients also showed decreased responsiveness to LA when cultured in normal human serum or fetal calf serum (data not shown). This is in accordance with the results of Williams et al in the rat system (12).

In conclusion the whole blood culture method for lymphocyte stimulation with LA is feasible and may provide useful information

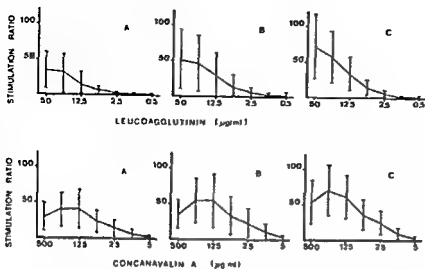


Fig 3 Lymphocyte stimulation with LA (above) and Con A (below) in 21 children with LUTI. The curves show mean stimulation ratios and the bars ± 1 SD of the mean. The dotted area represents ± 1 SD of the control group of children. The lymphocyte stimulation was studied at the acute stage (A), at 2 weeks (B) and at 6 weeks (C) after infection.

Lymphocyte stimulation in acute UTI At the acute stage of pyelonephritis the lymphocyte responses to LA were suppressed in all patients (< -1.0 SD of the control group) and the mean stimulation ratio of the group with the highest concentration of LA was below that of controls (15.7 vs 82.7 $p < 0.001$). The mean s.r. to Con A was similarly suppressed (Fig 2).

The shape of the dose response curve was also changed when maximal responsiveness was decreased. This was best seen in the Con A stimulated cultures at the acute stage of pyelonephritis as the best responses were obtained using Con A at a concentration of 500 $\mu\text{g/ml}$ which in normal conditions was inhibitory (Fig 2).

At 2 weeks the lymphocyte responses had become normal in approximately one half of the patients (Table 2) but the mean stimulation ratio of the group was still below control level. By 6 weeks there was no difference in the mean s.r. compared to controls (Fig 2) although 6 individuals showed impaired lymphocyte responsiveness at this stage.

In most patients with LUTI lymphocyte responses were suppressed in the acute stage (Table 2) but the mean stimulation ratio of the group was only approximately minus 1.0 standard deviations below the controls. There was some improvement of lymphocyte responsiveness by 2 weeks and by 6 weeks the mean stimulation ratio did not differ signifi-

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Lymphocyte stimulation in the level diagnosis of UTI Table 1 shows how the standard criteria for the diagnosis of pyelonephritis were met. In most cases there was no ambiguity in differentiating pyelonephritis from LUTI. Although there was no clear cut differences between the groups in the lymphocyte responsiveness it appeared that lymphocyte stimulation ratio below minus 2.0 standard deviations from the controls approaching energy was suggestive of pyelonephritis and correlated with the other criteria for systemic response to infection.

By 6 weeks at which time the initial infection had been treated in all cases there were still 13 patients in whom the lymphocyte stimulation ratio was below -1.0 SD of the control group. Among these patients 1 had recurrent pyelonephritis and 2 had a recurrent LUTI. Two more patients had another febrile infection. Four patients had X ray findings suggestive of chronic pyelonephritis and 1 had crossed ectopia of the right kidney. In the remaining 3 there was no apparent associated condition.

DISCUSSION

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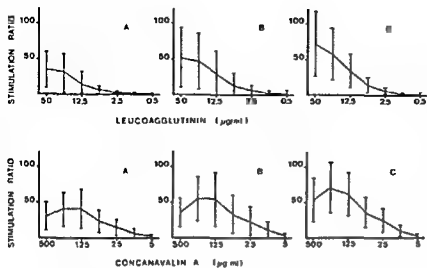


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The present results indicating suppression of CMI at the acute stage of urinary tract infections are supported by observations in animal

SEROLOGICAL DIFFERENTIATION OF CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTIONS DETECTED IN INFANCY

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ABSTRACT Ahlfors K. Ivarsson S. A. and Johnson T. (Department of Clinical Virology and Department of Paediatrics University of Lund Malmö General Hospital Malmö Sweden) Serological differentiation of congenital and acquired cytomegalovirus infections detected in infancy *Acta Paediatr Scand* 68 507 1979.—A serological investigation on infants with incidentally detected cytomegalovirus (CMV) excretion was made in an attempt to differentiate between congenital and acquired infections. Generally each of the patients 0-12 months old at the detection of CMV-excretion was studied by in direct immunofluorescence (IDF) test for IgM antibodies in cord serum and by complement fixation (CF) test performed on cord serum and a number of sera drawn after the detection of CMV. Two out of 4 patients with virologically confirmed congenital CMV infection as shown by positive virus isolation within 1 week of age had a positive CMV IgM test in cord serum. One of these two children also had a persistently high CMV CF titer from birth until 2 months of age indicating congenital infection. In the remaining 46 cases all with the CMV-excretion detected after 3 weeks of age in half of the cases after 5 months no positive IgM reaction was recorded in cord serum. No persistently high CF titer could be demonstrated among 13 out of the 46 patients from whom sera were drawn at birth and at 1-4 months. Six out of these 13 patients had a CF titer rise after the period of 1-4 months indicating acquired infection. However also one of the congenitally infected children had a similar titer increase. Many patients lacked characteristic serological patterns, some of them in spite of access to sera drawn at birth as well as at 1-4 months of age and later on. It could be concluded that the possibility of making a serological distinction of congenital and acquired infant CMV infection found by chance during infancy is limited.

KEY WORDS Cytomegalovirus infection serology congenital neonatal acquired

Because of frequent often unexpected finding of cytomegalovirus (CMV) infection among children treated at Malmö General Hospital there were two recurrent questions: first is the infection congenital with possible sequelae as result; second does the finding have any causal relationship to the patient's clinical symptoms. In order to analyse these questions 661 consecutive children 0-12 months old were studied by virus isolation at admittance to hospital (1). It was shown that CMV uria occurred among 1% of the infants less than one month old and among 23% of the older children. As a rule there was no clinical suspicion of CMV infection. From the virological studies it was concluded that CMV uria before 2-3 weeks of age could be considered proof of

congenital infection while thereafter it usually depended on natal/postnatal virus transmission. However in order to further analyse the patients with later detected infection serological studies were performed. The present study illustrates the difficulties in distinguishing between the congenitally and natally/postnatally infected CMV excretors found by chance in practical paediatric work.

MATERIAL AND METHODS

Four congenitally infected children Among the 661 patients studied four were shown to have CMV uria within the above mentioned critical period of 2-3 weeks of age in fact within one week. Only one of them had typical CMV symptoms at birth and neurological sequelae (1). Cord serum was drawn from each of these four patients and a total of 19 further sera were taken up to

of the patient's condition both at the acute and convalescent stages of UTI. Suppression of CMI appears to be of value in the level diagnosis of UTI. The role of cellular immune suppression in the pathogenesis of recurrent infections remains to be established.

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REFERENCES

- 1 Anttila R, Gröhn P & Krohn K. Transfer factor and cellular immune response in urinary tract infection in children. *Acta Paediatr Scand* 66: 219, 1977.
- 2 Buimovic Klein E, Vesikari T, Santangelo C F & Cooper L J. Study of the lymphocyte in vitro response to rubella antigen and phytohemagglutinin by a whole blood method. *Arch Virol* 57: 323, 1976.
- 3 Dubroff L M, Duckett J W & Corniere J N Jr. Phytohemagglutinin lymphocyte stimulation in children with recurrent urinary tract infections. *Urology* 5: 744, 1975.
- 4 Eskola J, Soppi E, Viljanen M & Ruuskanen O. A new micromethod for lymphocyte stimulation using whole blood. *Immunol Commun* 4: 297, 1975.
- 5 Hanson L Å, Ahlstedt S, Fasth A, Jodal U, Kauser H, Larsson P, Lindberg U, Olling S, Sohl Åkerlund A & Stanborg Edén C. Antigens of *Escherichia coli*: human immune response and the pathogenesis of urinary tract infections. *J Infect Dis* 136 (Suppl): 144, 1977.
- 6 Jodal U, Lindberg U & Lincoln K. Level diagnosis of symptomatic urinary tract infections in childhood. *Acta Paediatr Scand* 64: 201, 1975.
- 7 Kantor F S. Infection: energy and cell mediated immunity. *N Engl J Med* 297: 629, 1975.
- 8 Kouvalainen K, Pelkonen P & Parkkulanen K V. Immunoglobulins in chronic urinary tract infections in children. *Ann Clin Res* 1: 187, 1969.
- 9 Miller T E, Simpson G & Ormrod D J. Quantitation of immunoglobulin bearing lymphocytes and the lymphocyte response to PHA in experimental pyelonephritis. *Clin Exp Immunol* 21: 474, 1975.
- 10 Nilsson L Å. Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum. *Acta Pathol Microbiol Scand* 73: 179, 1968.
- 11 Park B H & Good R A. A new micromethod for evaluating lymphocyte responses to phytohemagglutinin. Quantitative analysis of the function of thymus dependent cells. *Proc Acad Sci USA* 69: 371, 1972.
- 12 Williams T W, Friedlander A M, Lyons J M & Brande A J. Cellular immunity in pyelonephritis: inhibition of the spleen cell response to the mitogen Concanavalin A. *Cell Immunol* 14: 33, 1974.
- 13 Winberg J. Determination of renal concentration capacity in infants and children without renal disease. *Acta Paediatr Scand* 48: 318, 1959.
- 14 Winberg J, Andersen H J, Bergstrom T, Jacobson H, Larson H & Lincoln K. Epidemiology of symptomatic urinary tract infection in childhood. *Acta Paediatr Scand* (Suppl) 252: 1, 1974.

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SEROLOGICAL DIFFERENTIATION OF CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTIONS DETECTED IN INFANCY

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ABSTRACT Ahlfors K, Ivarsson S A and Johnson T (Department of Clinical Virology and Department of Paediatrics University of Lund Malmö General Hospital Malmö Sweden) Serological differentiation of congenital and acquired cytomegalovirus infections detected in infancy *Acta Paediatr Scand* 68 507 1979.—A serological investigation on infants with incidentally detected cytomegalovirus (CMV) excretion was made in an attempt to differentiate between congenital and acquired infections. Generally each of the patients 0-12 months old in the detection of CMV excretion was studied by indirect immunofluorescence (IIF) test for IgM antibodies in cord serum and by complement fixation (CF) test performed on cord serum and a number of sera drawn after the detection of CMV. Two out of 4 patients with virologically confirmed congenital CMV infection as shown by positive virus isolation within 1 week of age had a positive CMV IgM test in cord serum. One of these two children also had a persistently high CMV CF titer from birth until 2 months of age indicating congenital infection. In the remaining 46 cases all with the CMV excretion detected after 3 weeks of age in half of the cases after 5 months no positive IgM reaction was recorded in cord serum. No persistently high CF titer could be demonstrated among 13 out of the 46 patients from whom sera were drawn at birth and at 1-4 months. Six out of these 13 patients had a CF titer rise after the period of 1-4 months indicating acquired infection. However, also one of the congenitally infected children had a similar titer increase. Many patients lacked characteristic serological patterns, some of them in spite of access to sera drawn at birth as well as at 1-4 months of age and later on. It could be concluded that the possibility of making a serological distinction of congenital and acquired infant CMV infection found by chance during infancy is limited.

KEY WORDS Cytomegalovirus infection serology congenital neonatal acquired

Because of frequent often unexpected finding of cytomegalovirus (CMV) infection among children treated at Malmö General Hospital there were two recurrent questions: first, is the infection congenital with possible sequelae as result; second, does the finding have any causal relationship to the patient's clinical symptoms. In order to analyse these questions 661 consecutive children 0-12 months old were studied by virus isolation at admittance to hospital (1). It was shown that CMV ura occurred among 1% of the infants less than one month old and among 23% of the older children. As a rule there was no clinical suspicion of CMV infection. From the virological studies it was concluded that CMV ura before 2-3 weeks of age could be considered proof of

congenital infection while thereafter it usually depended on natal/postnatal virus transmission. However, in order to further analyse the patients with later detected infection serological studies were performed. The present study illustrates the difficulties in distinguishing between the congenitally and natively/postnatally infected CMV excretors found by chance in practical paediatric work.

MATERIAL AND METHODS

Four congenitally infected children. Among the 661 patients studied, four were shown to have CMV ura within the above mentioned critical period of 2-3 weeks of age in fact within one week. Only one of them had typical CMV symptoms at birth and neurological sequelae (1). Cord serum was drawn from each of these four patients and a total of 19 further sera were taken up to

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REFERENCES

- Anttila R, Gruhn P & Krohn K. Transfer factor and cellular immune response in urinary tract infection in children. *Acta Paediatr Scand* 66: 219, 1977.
- Buimovic Klein F, Vesikari T, Santangelo C F & Cooper L Z. Study of the lymphocyte in vitro response to rubella antigen and phytohemagglutinin by a whole blood method. *Arch Virol* 57: 323, 1976.
- Dubroff I M, Duckett J W & Corniere J N Jr. Phytohemagglutinin lymphocyte stimulation in children with recurrent urinary tract infections. *Urology* 5: 744, 1975.
- Eskola J, Soppi E, Viikari M & Ruuskanen O. A new micromethod for lymphocyte stimulation using whole blood. *Immunol Commun* 4: 297, 1975.
- Hinson L Å, Ahlstedt S, Frith A, Jodal U, Krüger H, Larsson P, Lindberg U, Olling S, Sohl Åkerlund A & Svanborg Edén C. Antigens of *Escherichia coli*: human immune response and the pathogenesis of urinary tract infections. *J Infect Dis* 136 (Suppl. 1): 44, 1977.
- Jodal U, Lindberg U & Lincoln K. Level diagnosis of symptomatic urinary tract infections in childhood. *Acta Paediatr Scand* 64: 201, 1975.
- Kantor F S. Infection, energy and cell mediated immunity. *N Engl J Med* 297: 679, 1975.
- Kouvalainen K, Pelkonen P & Parkkainen K V. Immunoglobulins in chronic urinary tract infections in children. *Ann Clin Res* 1: 182, 1969.
- Miller T E, Simpson G & Ormrod D J. Quantitation of immunoglobulin bearing lymphocytes and the lymphocyte response to PHA in experimental pyelonephritis. *Clin Exp Immunol* 21: 474, 1975.
- Nilsson L Å. Comparative testing of precipitation methods for quantitation of C reactive protein in blood serum. *Acta Pathol Microbiol Scand* 73: 179, 1968.
- Park B H & Good R A. A new micromethod for evaluating lymphocyte responses to phytohemagglutinin. Quantitative analysis of the function of thymus dependent cells. *Proc Acad Sci USA* 69: 371, 1972.
- Williams T W, Friedlander A M, Lyons J M & Brande A J. Cellular immunity in pyelonephritis: inhibition of the spleen cell response to the mitogen Concanavalin A. *Cell Immunol* 14: 33, 1974.
- Winberg J. Determination of renal concentration capacity in infants and children without renal disease. *Acta Paediatr Scand* 48: 318, 1959.
- Winberg J, Andersen H J, Bergström T, Jacobson B, Larson H & Lincoln K. Epidemiology of symptomatic urinary tract infection in childhood. *Acta Paediatr Scand* Suppl. 252: 1, 1974.

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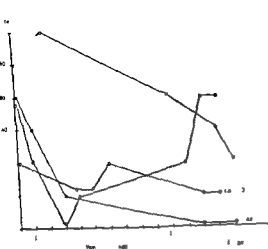


Fig 1 CF antibody development of congenitally infected patients

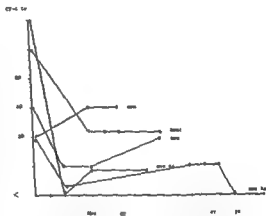
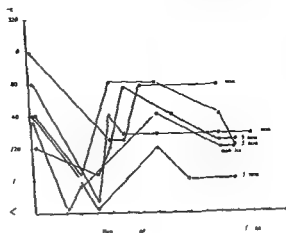


Fig 2 Individual CF titer development of patients with CMV infection detected after 3 weeks of age. The age of the patients at the detection of viraemia is noticed (A) Patients with CF titer rise (B) Patients without CF titer rise

mentioned fourth case the CF titer at 1-4 months of age was high (1/320) which was comparable to the maternal titer 1/160 at delivery. After the period of 1-4 months two patients had falling levels which in one case ended in a titer $\leq 1/5$ one patient had a persistently low titer (1/20-1/10) and another one a marked CF titer rise.

CF antibodies among infants with CMV viraemia detected after 3 weeks of age. At birth 43/45 (96%) patients tested had a positive CMV CF titer varying between 1/10-1/320 with the geometric mean (GM) titer 1/40. The CF titer development of the two patients with negative test (CF titer $\leq 1/5$) in cord serum born by CF negative mothers was not followed up. Virus isolations from these two patients were positive in the fourth week and at ten months of age. On comparison of sera drawn at birth and at 1-4 months 11/13 infants with CMV excretion detected at 1-4 months of age had falling titers while the remaining two showed persistently low titers ($\leq 1/40$). After the age of four months six patients with CMV viraemia at 1-4 months showed significant titer increases (Fig 2A). Five other patients with primary CMV isolation at 1-10 months and sera drawn at 1-4 months and later on did not show any postnatal titer rise (Fig 2B). For the 19 children

admitted to hospital after five months of age sera drawn at 1-4 months and in the acute phase of the disease usually were missing. In spite of continued virus excretion five patients showed a significant CF titer decrease after 7-8 months of age, one of them to titer $\leq 1/5$. At 2-3 years 4/16 (25%) of the children tested did not have measurable amounts of CF antibodies.

CF antibodies among mothers of congenitally infected children. The distribution of maternal CMV CF titers at delivery is in this and the two following paragraphs is shown in Fig 3. All mothers of the congenitally infected infants had positive CF titers at the

3 years of age. The cord sera were studied by indirect immunofluorescence (IIF) test for IgM antibodies and all sera but one cord sample by complement fixation (CF) test.

Forty six children with CMV uria detected after 3 weeks of age. Out of the 661 infants tested 57 were more than 3 weeks old at the detection of CMV uria almost half of them in fact more than 5 months. Only forty six of them were studied by serological methods. Different symptoms of infection predominated in the entire patient material as well as among the CMV excretors (1). Forty five cord sera were taken and 103 sera from one month up to three years of age 1-8 per individual. Few postnatal sera were taken until the virus isolation result was evident. The cord sera were—with few exceptions—tested by IgM IIF and all sera by CI.

Randomized cord sera for control of the IgM IIF technique. One hundred cord sera from randomized babies were tested by the IgM IIF technique for specific and unspecific reactions. The clinical status of these babies was not analysed.

Serum panel for control of the CI technique. A panel of 14 sera kindly supplied by professor U. H. Krech-Schweiz was tested by CF at 14 laboratories the present one included.

Mothers of the four congenitally infected children. In all cases maternal sera were drawn at delivery. The samples were tested by CF.

Mothers of the 46 children with CMV uria detected after 3 weeks of age. Sera were drawn from 42/46 mothers at delivery. They were studied by CF.

Control group of 348 randomized mothers. This group was studied in order to obtain information on the CMV CF status at delivery in general.

Sera. Sera had been stored for a varying period of time up to five years at -20°C .

Indirect immunofluorescence test for IgM antibodies in cord sera. Slide cultures of human embryonal lung fibroblasts grown in Eagle's MEM with 10% fetal calf serum and antibiotics and maintained in Eagle's MEM with 3% fetal calf serum and antibiotics were inoculated with CMV AD 169 incubated in CO_2 atmosphere at 35°C and 3-5 days after the appearance of cytopathogenic effect washed in phosphate buffer and fixed in acetone at 4°C for 10 min. The slides were stored at -20°C for at least one week before being tested. They were stable for months. Before use they were washed in distilled water. Cord sera were tested concomitantly on two different batches of slides at dilutions 1/4 and 1/16 and incubated at 35°C for 3 hours. Two positive serum controls, one at low dilution giving a strong fluorescence and one at high dilution near the positive end point were included on each slide together with a negative serum control with a high CMV IgG titer. After a rapid rinsing in phosphate buffer pH 7.2 followed by a thorough washing with magnetic stirrer in the same type of buffer and a short rinsing in distilled water the slides were incubated for 1 hour with a specific FITC conjugated rabbit anti human (anti IgM heavy chains) serum from Dakopatts A/S Copenhagen Denmark diluted 1/15. After washing as before the slides were mounted in glycerine buffer pH 7.8. A Leitz fluorescence microscope

with transmitted light was used for reading. Only the fluorescence of intranuclear inclusions graded from 1+ to 3+ was recorded (8-13). Sera positive at the screening were retested at two-fold serial dilutions.

Test for rheuma factors. IgM IIF positive sera were tested for rheuma factors (RF) with the Latex RF Reagenz Behringwerke Germany at dilution 1/1, 1/4, 1/8, 1/16, 1/32, 1/64.

Complement fixation test. CMV CF test which mainly reflects the IgG antibody status (3, 4, 6, 7) was performed by a microtiter technique using 4-6 units of sonicated and heated (56°C 60 min) CMV AD-169 crude cell extract antigen and 2 units of complement. Complement pretreatment was performed on all sera in order to prevent anticomplementary activity which was a common phenomenon (17). A fourfold CF titer increase or decrease was considered significant. Titers $\leq 1/5$ negative and $\geq 1/10$ positive. Maternal and infant sera were tested in the same CF run.

RESULTS

IgM antibodies in cord sera from congenitally infected children. Two out of the four congenitally infected children had a positive IgM IIF reaction in cord serum both with titers 1/32. Both were RF negative when tested at serum dilution 1/2 and 1/20 respectively. One of the IgM positive children had a typical CMV disease the other one was asymptomatic.

IgM antibodies in cord sera from infants with CMV uria detected after 3 weeks of age. Thirty nine out of the 46 children in this group were tested for CMV IgM antibodies in cord serum. In all cases the IgM IIF titer was negative that is $< 1/4$.

Randomized cord sera for control of the IgM IIF technique. All 100 randomized cord sera had CMV IgM titers $< 1/4$. The cell nuclei appeared as black holes in a faint fluorescing cytoplasm.

CF antibody development among congenitally infected children. Three patients had a positive CMV CF titer in cord serum varying between 1/20-1/80. In the fourth case no CF test was performed (Fig. 1). On comparison of cord sera and samples drawn at 1-4 months of age two patients showed significantly falling titers and the third a persistently low titer (1/20-1/10). In the above

noted by others (2, 9, 16). It is probable that the mother is the source not only of the congenital but also of most acquired infant infections.

From the paper by Stagno et al. it is evident that studies of the infant CF antibody development should include a serum sample drawn at 1-4 months of age (15). The average CF antibody titer of congenitally infected patients is then comparatively high in contrast to that of neonatally infected babies. However, as shown in the present study, only one out of the four congenitally infected infants followed the expected pattern (Case 1, Fig. 1). Born to a mother with CF titer 1/160 and with its own CF titer 1/320 at 2 months of age, this child probably had had a persistently high titer since birth. It might be of interest to add that this child was the only one who was symptomatic at birth and developed sequelae. The absence of persistently high titers among patients with supposedly acquired infection does not exclude the possibility that some of them were congenitally infected as shown above.

Six patients with CMV infection detected after 3 weeks of age had a postnatal CF titer rise, a pattern which, according to the previous observations by Stagno et al., should be typical for acquired infection (15). However, since also one of the congenitally infected patients in the present material—and two patients in the material of Melish & Hanshaw (11)—had such titer increases, there obviously are exceptions to the rule. Five other patients with supposed acquired infection and adequately drawn sera did not have postnatal titer increase. The connection between titer rise and current disease in the above mentioned cases is an open question.

Follow up until 3 years of age showed that 5/20 (25%) of the patients tested—one with congenital and four with probably acquired infections—lost their CF antibodies. Thus, a negative CF test performed on an older child or an adult person does not exclude the possibility of previous CMV infection and CF

seroconversion later in life does not necessarily mean primary CMV infection. Possibly more sensitive techniques like the enzyme linked immunosorbent assay might have shown residual antibodies.

In summary, only two out of the four cases with confirmed congenital infection would have been diagnosed by the demonstration of specific IgM antibodies in cord serum. In one of these cases the diagnosis would have been indicated by a persistently high CF titer at 1-4 months of age. In 6/46 cases with supposed acquired CMV infection the proposed diagnosis was supported by a postnatal CF titer rise. In the remaining 40 cases the type of infection remained doubtful, in some cases because of the absence of typical serological patterns, specific IgM antibodies at birth, persistently high CF antibody titers from birth until 1-4 months or CF titer increases. In other cases sera drawn at the proper times were missing. The present study has shown that the possibility of a serological diagnosis of congenital CMV infection is rather limited, especially if cord serum is not available.

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REFERENCES

1. Ahlfors K, Ivarsson B, A. Johansson T & Svensson I. Congenital and acquired cytomegalovirus infections. Virological and clinical studies on a Swedish infant population. *Acta Paediatr Scand* 67: 321, 1978.
2. Andersen H, K. Gravesen J, J. & Iversen T. Cytomegalovirus infection among infants admitted to a paediatric department. *Acta Paediatr Scand* 61: 445, 1972.
3. Bernstein M, T. & Stewart J, A. Indirect hemagglutination test for detection of antibodies to cytomegalovirus. *Appl Microbiol* 21: 84, 1971.
4. Cremer N, E. Schmidt N, I. Jensen F, Hoffmann M, Oshiro I, S. & Lenette E, H. Complement fixing antibody in human sera reactive with viral and soluble antigens of cytomegalovirus. *J Clin Microbiol* 11: 67, 1975.

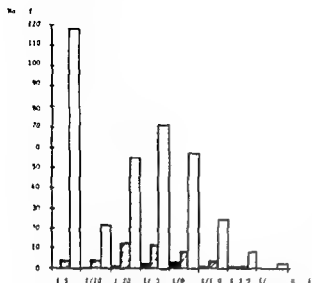


Fig. 3 Distribution of CF titers at delivery among mothers of congenitally infected children ■ mothers of children with probable perinatal/acquired infection ▨ and randomized control mothers □

delivery with the GM titer 1/80. In the three cases studied the maternal and infant titers were equal at birth (a difference of \leq one titer step).

CF antibodies among mothers of children with CMV vira detected after 3 weeks of age. Thirty nine out of 42 (93%) mothers tested had positive CMV CF reactions at the delivery with the GM titer 1/40. In 4/37 cases tested the infant titer level at birth exceeded the maternal titer with two steps while in the other 33 cases the titers were equal.

CF antibodies in a control group of randomized mothers. Among 348 mothers tested 237 (68%) had positive CMV CF reactions at delivery with the GM titer 1/40.

Serum panel for control of the CF technique. According to a compilation by professor Krech there was almost complete agreement (95%) between the different laboratories regarding the outcome of the CMV CF tests—positive or negative. In no case did the outcome of the present laboratory diverge from the common results. The highest titers were obtained at this and five other laboratories.

DISCUSSION

In the present serological investigation the starting point was a positive CMV isolation

from a patient at any time between birth and one year of age. Access to frozen cord sera was of primary importance. More sera drawn at 1–4 months and in the acute stage of the disease would have been desirable. Previous serological studies on CMV infected infants have mainly been prospective starting directly after birth (11–15). Thus Melish & Hinshaw (11) studied the occurrence of CMV IgM antibodies among a series of newborn infants and the CF titer development of the congenitally infected babies detected. Stagno *et al* (15) followed from birth the antibody development of infants congenitally or neonatally infected by CMV.

In the present study only two out of the four congenitally infected children—the diagnoses confirmed by positive virus isolation within one week of age—had positive IgM IIF tests at birth as a serological sign of the intrauterine infection. One of these two patients was symptomatic at birth, the other asymptomatic. It is well known that congenitally infected children often do not have CMV IgM antibodies at birth (10–11). According to Luthardt (10) there is no correlation between the occurrence of IgM antibodies and the gravity of the disease. It can be concluded that some children with CMV vira detected after 3 weeks of age can have been congenitally infected in spite of the absence of positive IgM reactions at birth.

Determination of the infant—or the maternal—CF antibody status at birth was shown to be of little differential diagnostic value since all congenitally infected infants tested as well as 43/45 (96%) of those with probable acquired infection had a positive reaction. When the mothers of the children with suspected acquired infection were compared to mothers in the general population 39/42 (93%) were CMV CF positive in the former group and 237/348 (68%) in the latter group—a difference which is statistically significant ($p < 0.001$). The above mentioned finding that children infected by CMV as a rule are born to seropositive mothers had already been

noted by others (2 9 16) It is probable that the mother is the source not only of the congenital but also of most acquired infant infections

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REFERENCES

- 1 Ahlfors K Ivarsson M A Johnsson T & Svensson I Congenital and acquired cytomegalovirus infections Virological and clinical studies on a Swedish infant population *Acta Paediatr Scand* 67 371 1978
- 2 Andersen H K Gravesen J J & Iversen T Cytomegalovirus infection among infants admitted to a paediatric department *Acta Paediatr Scand* 61 445 1972
- 3 Bernstein M T & Stewart J A Indirect hemagglutination test for detection of antibodies to cytomegalovirus *Appl Microbiol* 21 84 1971
- 4 Cremer N E Schmidt N J Jensen E Hoffmann M Oshiro L S & Lenette E H Complement fixing antibody in human sera reactive with viral and soluble antigens of cytomegalovirus *J Clin Microbiol* 1 762 1973

- 5 Fraser K B Shirodaria P V & Stanford C F Fluorescent staining and human IgM *Br Med J* III 707 1971
- 6 Hanshaw J H Congenital cytomegalovirus infection laboratory methods of detection *J Pediatr* 75 1179 1969
- 7 Lang D J & Noren B Cytomegaloviremia following congenital infection *J Pediatr* 73 812 1968
- 8 Langenhuisen M M A C The T H Niewek H O & Kapsenberg J G Demonstration of IgM cytomegalovirus antibodies as an aid to early diagnosis in adults *Clin Exp Immunol* 6 387 1970
- 9 Leimikki P Granstrom M L Santavuori P & Peltty O Epidemiology of cytomegalovirus infections during pregnancy and infancy A prospective study *Scand J Infect Dis* 10 165 1978
- 10 Luthardt T Cytomegalie In O Vivell & W Burmeister (eds) *Klinische Paediatric Beihefte Ferdinand Enke Verlag Stuttgart* 1976 p 14
- 11 Melish M E & Hanshaw J B Congenital cytomegalovirus infection Developmental progress of infants detected by routine screening *Am J Dis Child* 126 190 1973
- 12 Reimer C B Balck C M Philips D J Logan L C Hunter F F Pender B J & McGrew H F The specificity of fetal IgM antibody or anti antibody? *Ann NY Acad Sci* 254 77 1975
- 13 Schmitz H & Haas R Determination of different cytomegalovirus immunoglobulins (IgG IgA IgM) by immunofluorescence *Arch Gesamte Virusforsch* 37 131 1972
- 14 Shirodaria P V Fraser K B & Stanford F Secondary fluorescent staining of virus antigens by rheumatoid factor and fluorescein conjugated anti IgM *Ann Rheum Dis* 32 53 1973
- 15 Stagno S Reynolds D W Tsiantos A Fucillo D A Long W & Alford C A Comparative serial virologic and serologic studies of symptomatic and subclinical congenitally and natively acquired cytomegalovirus infections *J Infect Dis* 137 568 1975
- 16 Stoll H U Zytomegalievirusausscheidung bei gesunden Kindern im ersten Lebensjahr *Schweiz Med Wochenschr* 104 468 1974
- 17 Taran A A simple method for performing a Wassermann test on anticomplementary serum *J Lab Clin Med* 31 1037 1946

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CLINICAL FINDINGS AND INTESTINAL IMMUNOGLOBULINS
IN CHILDREN WITH PARTIAL IgA DEFICIENCY

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ABSTRACT Savilahti E. and Pelkonen P. (Children's Hospital, University of Helsinki, Helsinki, Finland). Clinical findings and intestinal immunoglobulins in children with partial IgA deficiency. *Acta Paediatr Scand* 68: 513, 1979. —We studied the intestinal morphology and the jejunal and rectal immunoglobulins of 16 children with partial IgA deficiency defined as serum IgA concentration more than two standard deviations below the mean for age but higher than the lower limit of sensitivity of single radial immunodiffusion (0.02 g/l). Five of the patients had been treated with phenytoin. 2 had juvenile rheumatoid arthritis. 2 had ulcerative colitis and 5 had recurrent upper respiratory tract infections. The jejunal morphology was normal in every case. In 6 cases normalization of serum IgA occurred during the follow-up, while in one patient with ulcerative colitis the concentration fell below 0.02 g/l. In patients with recurrent infections there was a decreased frequency of infections when the level of serum IgA increased. In 4 patients IgM-containing cells predominated in both the jejunal and rectal mucosa, and IgM was increased in the intestinal juice. In 6 patients a significant increase in IgM-containing cells or a decrease in IgA-containing cells or both were seen in either the rectal or jejunal mucosa. There was no correlation between the number of IgA-containing cells in the intestinal mucosa and the serum level of IgA.

KEY WORDS Partial IgA deficiency, low serum IgA, intestinal immunoglobulins

Selective deficiency of IgA is a well-characterized and common immunodeficiency (2, 8, 12, 21). In addition to the deficiency of IgA in serum and secretions, there is replacement of IgA by IgM in the mucous membranes and secretions (5, 8, 23). The clinical features of IgA deficiency are well known. Much less is known about the importance of partial IgA deficiency, its clinical associations and mucosal immunoglobulins. In recent years there have been clinical reports of patients with a decrease in serum IgA attributed to treatment with phenytoin (1, 3, 24) and *d*-penicillamine (10). These patients have had low but measurable quantities of serum IgA. This report is based on our experience with children having a partial deficiency of serum IgA. In addition to clinical characteristics, we have studied mucosal immunoglobulins in the intestine of 16 such patients. Most of them have been followed for several years, and changes in the

serum and rectal immunoglobulins have been documented.

MATERIALS

The study includes 16 children over 1 year of age whose serum level of IgA was more than two SD below the mean for age but above the lower limit of sensitivity of single radial immunodiffusion (0.07 g/l) (Fig. 1). They had normal or elevated levels of IgG, IgM and IgE. The patients were detected among 3 000 children over 2 years of age whose sera were sent to routine immunoelectrophoretic study in 1972-73 in the Children's Hospital, University of Helsinki. During the same period 12 subjects with selective IgA deficiency (serum concentration below 0.00 g/l) were found.

The results on the intestinal immunoglobulins are compared with those of immunologically normal children (7) and children with selective IgA deficiency (3) studied by the same method.

METHODS

Serum concentrations of IgA, IgG and IgM were measured by the single radial immunodiffusion method and compared with normal values of children established in

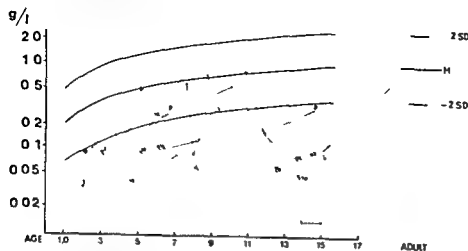


Fig. 1 Serum IgA levels of the patients. Mean (M), +2SD and -2SD for age are indicated (II). The time of the intestinal studies and of the follow up rectal biopsies is given (I). The case numbers correspond to those in Table 1.

the same laboratory (11). The antisera of IgA, IgG and IgM used were products of Hyland Laboratories. Our serum standard has been originally standardized using a serum sample which had been assayed for the immunoglobulin concentrations in the laboratory of Dr D. Gitlin, Pittsburgh, PA. The serum standard has subsequently been calibrated against the WHO reference preparation 67/97 (18) and the transformation coefficients are IgA 1 IU = 0.88 mg, IgM 1 IU = 0.60 mg and IgG 1 IU = 9.1 mg. IgF was measured with the commercial radioimmunoassay kit Elidelab[®] (Pharmacia, Sweden). Antibodies to cow's milk and gluten were studied by a double diffusion micromethod (11). Intestinal juice was collected via duodenal intubation. Its content of IgA, IgM and IgG was measured by electroimmunodiffusion (22).

Small intestinal biopsy specimens were taken from the upper jejunum with a Crosby-Kugler biopsy capsule. The specimens were studied under a dissecting microscope and divided for light microscopy and immunofluorescence study. In addition, a part of 11 biopsy specimens was used for the measurements of maltase, sucrase, lactase and isomaltase activities (20). The morphology was examined as described by Kuitunen (13). Tissue for the immunofluorescence study was processed and stained with FITC conjugated anti-IgA, IgM, IgG, IgE and IgD antisera as described earlier (23). The number of positively stained cells in an area of at least 0.2 mm² between surface epithelium and muscularis mucosae was counted directly on the slides at 1000 fold magnification under a Leitz microscope with epillumination. A mercury vapour lamp was used as the light source with K 495 and K 530 barrier filters (17).

RESULTS

Clinical findings and follow up of IgA levels

The intestinal studies were performed at or shortly after the time the partial IgA deficiency was detected (Fig. 1). Patients 1-5 (Table 1) had received phenytoin treatment prior to the finding of a low IgA value. In patients 2, 4

and 5 a normal serum IgA had been documented before phenytoin treatment was started (Fig. 1). In cases 1-3 a control sample taken after discontinuation of the treatment showed an elevation of the IgA level but it was still more than 2 SD below the mean for age in case 3. In case 4 there was some increase in IgA in spite of continued phenytoin treatment. In the two patients with ulcerative colitis (cases 8 and 9) a normal IgA was recorded in the early phase of the disease (Fig. 1). Both were treated with Salazopyrin[®]. The older child had also chronic active hepatitis and received at times a small dose of prednisone. His IgA level fell to a very low value (below 0.02 g/l) and remained undetectable. In both cases with juvenile rheumatoid arthritis (JRA) we saw normalization of serum IgA during the follow up period (Table 1). Three children had atopic disease and frequent respiratory tract infections. All had been treated with anticongestants prior to the finding of low IgA (Table 1). In case 12 a normal IgA was recorded before commencement of medication.

Levels of IgG, IgM and IgE. One patient had an IgG level more than 2 SD below the mean for age at the initial examination but it rose during the follow up (case 16 in Table 1). In patient 7 with JRA increased concentrations of IgG were observed. The IgM levels of the patients were within the normal range. Two patients had elevated levels of IgF and both of them had atopic symptoms.

Table 1 *Clinical and immunological data*

Case	Age at examination (years)	Clinical diagnosis	Medication (age period)	Clinical state at follow up	Serum immunoglobulins (g/l)			Precipitating antibodies to cow's milk
					IgA	IgG	IgM	
1	6.6	RURTI febrile convulsions	Phenytoin (0.7-4.0)	Improved	0.03 0.26	6.5 11.1	0.34 0.41	-
2	3.1 7.7	RURTI febrile convulsions	Phenytoin (1.7-4.5)	Improved	0.08 0.80	9.7 13.4	1.32 0.71	-
3	14.5 17.7	Abdominal pains	Phenytoin (9.5-14.6)	Asymptomatic	0.08 0.6	10.0 8.5	0.97 0.56	+
4	10.4 14.4	RURTI febrile convulsions	Phenytoin (9.5-cont.)	Improved	0.17 0.30	17.4 17.0	0.48 0.59	+
5	13.6	Convulsions	Phenytoin (10.2-cont.)	-	0.08	10.5	1.40	-
6	2.7 5.0	Rheumatoid arthritis	Chloroquine (1.5-cont.) Salicylates (3.7-3.7)	Inactive JRA	0.06 0.43	6.4 17.6	0.05 1.00	-
7	15.0 18.7	Rheumatoid arthritis	Chloroquine (9.5-17.1) Myochrysine (11.8-cont.)	Active JRA	0.09 0.66	78.7 36.6	1.30 1.45	+
8	11 10.7	Ulcerative colitis	Salazopyrin (7.5-cont.)	Asymptomatic	0.13 0.78	14.7 11.7	0.56 0.41	-
9	1.5 15.5	Ulcerative colitis chronic active hepatitis	Prednisolone (10-14) Salazopyrin (10.7-14.0)	Asymptomatic	0.05 ~0.07	7.1 29.3	0.40 1.42	+
10	13.7 16.9	Diabetes mellitus thyroiditis	Insulin (17.1-cont.) Thyroxine (1.6-cont.)	Unchanged	0.05 0.33	1.1 13.1	0.55 0.51	-
11	14.3	Acute nephritis	-	-	0.14	13.0	0.97	-
12	4.7 7.1	RURTI	Anticongestant (4.5-cont.)	Improved	0.03 0.08	7.5 9	0.56 0.37	+
13	5.4 9.4	Allergic rhinitis RURTI	Anticongestant (3-6)	Improved	0.08 0.32	10.2 12.6	0.68 0.38	-
14	8.4	Bronchial asthma	Anticongestant (7-8.4)	-	0.06	10.1	0.52	-
15	5.9 9.9	Dystonic tetraplegia athetosis	Diazepam (7.1-cont.)	Improved	0.77 0.67	15.4 13.4	0.70 0.69	+
16	6.3 8.1	Urinary tract infections	Sulfisoxazole (6.3-8.0)	Asymptomatic	0.11 0.17	5.7 7.5	0.50 0.44	-

RURTI=recurrent upper respiratory tract infections. Anticongestant¹ contains phenylephrine, chlorpheniramine and mepyrmine. Anticongestant² contains phenylpropanolamine and cinnarizine.

Serum antibody studies: Antibodies to gluten were not found in any of the cases, but six of the patients had precipitating antibodies to cow's milk (Table 1). Four of the six had abnormal cell counts in their jejunal mucosa (Fig. 2); one had a tendency to tracheal aspiration on account of his neurological disease.

Intestinal morphology and disaccharidase activities: The morphology of the jejunal biopsy specimens was normal in all 16 cases. In one patient lactase deficiency was found (lactase activity 6 U/g; patient 3, normal val-

ues >20 U/g). He had been treated with phenytoin because of suspected abdominal epilepsy. Normal disaccharidase activities were found in the other 10 cases studied. Patient 8 with ulcerative colitis had inflammatory changes in the rectal biopsy specimen; all the other rectal biopsies were morphologically normal.

Immunoglobulin-containing cells in intestinal mucosa: The total numbers of immunoglobulin-containing cells were very similar in immunologically normal children, patients

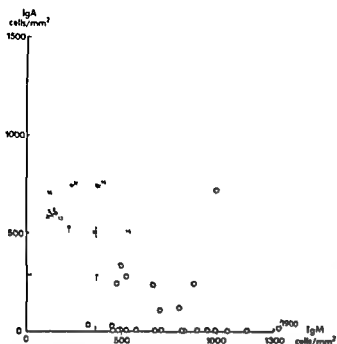


Fig. 2 Numbers of IgA and IgM-containing cells in the jejunal mucosa of patients (x and case numbers as in Table 1), immunologically normal children (o) and children with selective IgA deficiency (o). The mean -2 S.D. for IgA and the mean $+2$ S.D. for IgM cell numbers of the normal children are indicated.

with low serum IgA and patients with selective IgA deficiency (Table 2). The number of IgA and IgM containing cells in the individual cases in this study, in children with normal immunoglobulins and in patients with selective IgA deficiency are given for jejunal mucosa in Fig. 2 and for rectal mucosa in Fig. 3. In 14 biopsy specimens of 10 patients the number and ratio of IgA and IgM containing cells was abnormal, defined as a decrease of IgA containing cells to a number less than the mean

-2 S.D. of normals or an increase of IgM cells to a number more than the mean $+2$ S.D. of normals or the presence of more IgM than IgA cells. Four patients had abnormal results in both jejunal and rectal biopsies (cases 7, 9, 12, 14); in 6 patients the cell count was abnormal in one of these tissues and 6 patients had findings within 2 standard deviations of the controls. No correlation between the serum IgA level and the number of IgA-containing cells in the intestinal mucosa could be found. In individual cases the alteration in the mucosal immunoglobulins was unpredictable, e.g. the patient with the highest serum IgA (case 15) had an abnormal cell count in the jejunal mucosa and the finding in patient 10 with a low serum IgA value (0.05 g/l) was normal in both biopsy specimens.

The patients had significantly more IgG containing cells in both the rectal ($p < 0.05$) and the jejunal mucosa ($p < 0.01$) than immunologically normal children (Table 2) and also more than patients with selective IgA deficiency. IgE- and IgD containing cells were equally rare in the biopsy specimens from both groups of patients and from normal children.

In five patients a rectal biopsy specimen was studied in connection with the follow up (Fig. 1). All had normal numbers of immunoglobulin containing cells. In two (cases 1 and 3) the finding had been abnormal in the earlier biopsy specimen.

Immunoglobulins in the intestinal juice. On the average patients with low serum IgA had

Table 2 Total numbers of immunoglobulin containing cells and IgG containing cells in the intestinal mucosa

Intestinal mucosa						
	No of cases	Jejunal mucosa cells/mm ²		No of cases	Rectal mucosa cells/mm ²	
		Mean \pm S D			Mean \pm S D	
		Total	IgG		Total	IgG
Patients with partial IgA deficiency	16	970 \pm 176	101 \pm 61	15	609 \pm 231	103 \pm 81
Children with normal serum immunoglobulins	17	1 030 \pm 331	53 \pm 26	12	619 \pm 188	20 \pm 17
Patients with selective IgA deficiency	25	931 \pm 379	83 \pm 51	24	448 \pm 245	36 \pm 35

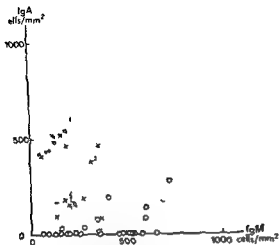


Fig. 3 Numbers of IgA and IgM-containing cells in the rectal mucosa. Symbols as in Fig. 7

only slightly more IgM and less IgA in the intestinal juice than children with normal immunoglobulins (Table 3). The concentration of IgM was higher than that of IgA in 8 samples from 15 patients; in only one of these was the number of IgA- and IgM-containing cells normal in the biopsy specimens. This ratio in the intestinal juice was seen in 3 out of 15 children with normal serum immunoglobulins.

DISCUSSION

It is well established that immunologically normal persons have a predominance of IgA-containing cells in their intestinal mucosa (7, 19, 22). In selective IgA deficiency this abundant IgA cell population is replaced by IgM-containing cells (8, 23). However, in a study of a large group of IgA-deficient subjects some of

them were found to have significant numbers of IgA-containing mucosal cells and these patients also had some IgA in their sera (23). In the present series of patients with partial IgA deficiency we found that they had normal total numbers of immunoglobulin-containing cells and 6 of them had normal ratios of IgA and IgM-containing cells. However, 10 patients showed either an increase in IgM-containing cells or a decrease of IgA-containing cells or both. This change from the normal cell ratio varied in degree and in some of the patients it was evident only in either the jejunal or the rectal specimens, whereas in others the finding was similar to that found in selective IgA deficiency. No correlation between the serum level of IgA and the cell numbers could be found. With regard to mucosal immunoglobulin production, patients with partial IgA deficiency form a transitional group between persons with normal serum immunoglobulins and patients with selective IgA deficiency; the mucosal immunoglobulins are unpredictable on the basis of the serum level of an individual patient. The situation is compatible with a varying degree of suppression of IgA production by T cells (25) resulting in disproportion in serum level of IgA and the number of IgA-containing cells in the intestine. Similar dichotomy between serum and gut immunoglobulins has been described in patients with hypogammaglobulinaemia (4).

A greater number of IgG-containing cells were noted in the mucosal samples than in earlier group studies (22, 23). The difference may be partly explained by technical differences, since during this study a change was made to epillumination and thus facilitated the dis-

Table 3 Immunoglobulins in the intestinal juice

	No. of samples	IgA g/l (mean \pm S.D.)	IgM g/l (mean \pm S.D.)
Patients with partial IgA deficiency	15	0.073 \pm 0.017	0.025 \pm 0.015
Children with normal serum immunoglobulins	15	0.047 \pm 0.018	0.021 \pm 0.017
Patients with selective IgA deficiency	24	Not calculated	0.080 \pm 0.041

tion of IgG-containing cells from the brick ground. Specimens of 14 patients in this study were measured with epifluorescence.

The association of low serum IgA and phenytoin treatment has been previously verified (13, 24). Five of our patients were on phenytoin at the time when the low serum IgA was noted. A normal pretreatment level of serum IgA was found in three cases and normalization of the level occurred after discontinuation of the drug in three other cases. In addition to phenytoin, *d*-penicillamine has been shown to depress serum IgA (10). On the basis of our study, another group of drugs can be suspected of exerting a similar effect, namely anticongestants containing antihistamines and vasoconstrictive agents. Three of the patients were receiving these drugs all on a long term basis. In one we found a normal IgA level prior to anticongestant therapy and in another normalization of IgA level took place after discontinuation of the treatment.

In the course of this study we found two patients with low serum IgA and JRA. In an analysis of a large group of children with JRA 19 cases with decreased and also deficient serum IgA were discovered (16), 9 of which turned out to be transient. A transient depression of IgA was seen in one of two patients with ulcerative colitis (UC) while in the other case IgA gradually fell and total IgA deficiency developed. A similar decrease of IgA has been previously described in a patient with chronic active hepatitis (14) and in one case of monozygotic twins after intestinal infection (15). The cases with JRA and UC in this study have been treated with multiple drugs and one may speculate whether the changes in serum IgA are due to drug treatment or due in part to the primary disease.

Our series includes four patients presenting with recurrent upper respiratory tract infections and two with congenital neurological disease. It is possible that some of these patients have a genetically determined alteration in the production of IgA, since two patients have a stable low level of IgA.

In at least 5 patients low serum IgA was documented during a period of frequent respiratory infections. In every one of these cases infections became more rare as serum IgA increased, suggesting the importance of secretory IgA in the defence of mucosal membranes. Although many persons with selective IgA deficiency are in good health (12) respiratory infections are the presenting symptom in a large number of cases with this deficiency (2, 21). Local immunoglobulins in the gut are important in protection against absorption of ingested native proteins from the intestine of rats (26). In man this antigen exclusion is probably provided by secretory IgA (26) e.g. human milk has secretory IgA antibodies to cow's milk proteins which are capable of reducing antibody response to cow's milk in infants (9). As a sign of defective antigen exclusion most persons with IgA deficiency have precipitating antibodies to cow's milk proteins (6, 21); in this group they were also more common than in controls and their presence was associated in most cases with a decrease in jejunal IgA containing cells. The frequency of coeliac disease is much increased in selective IgA deficiency (21) and up to date we have found 7 children with coeliac disease out of 74 with a selective IgA deficiency in whom jejunal biopsy had been performed. In children with partial IgA deficiency we did not find a single case with abnormal jejunal mucosa; therefore we have come to the conclusion that performing an intestinal biopsy on asymptomatic children with low serum IgA is not indicated.

We feel that it is of clinical importance to make a clear distinction between partial and total IgA deficiency. Partial IgA deficiency is in most cases a transient phenomenon followed by an increase in IgA, though in one of our cases an acquired total deficiency of IgA resulted. Partial IgA deficiency is often seen in connection with various drug regimens and in that case discontinuation of the drug should be considered. In patients with partial IgA deficiency mucosal immunoglobulins can

belong predominantly to either the IgA or the IgM class

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REFERENCES

- 1 Aarli J A & Tonder O Effect of antiepileptic drugs on serum and salivary IgA *Scand J Immunol* 4 391 1975
- 2 Ammann A J & Hong R Selective IgA deficiency Presentation of 30 cases and a review of the literature *Medicine* 50 13 1971
- 3 Andersen P & Mosekilde L Immunoglobulin levels and autoantibodies in epileptics on long term anticonvulsant therapy *Acta Med Scand* 210 69 1977
- 4 Broom B C de la Concha E G Webster A D Loewi G & Asherson G L Dichotomy between immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinaemia *Lancet* ii 53 1975
- 5 Brandtzaeg P Fjellander I & Gjeruldsen S T Immunoglobulin M Local synthesis and selective secretion in patients with immunoglobulin A deficiency *Science* 160 789 1968
- 6 Buckley H & Dees S C Correlation of milk precipitins with IgA deficiency *Br Med J* 781 465 1969
- 7 Crabbé P A Carbonara A O & Heremans J F The normal human intestinal mucosa as a major source of plasma cells containing γ A immunoglobulin *Lab Invest* 14 35 1965
- 8 Crabbé P A & Heremans J F Lack of gamma A-immunoglobulin in serum of patients with steatorrhea *Gut* 7 119 1966
- 9 Hanson L Å Ahlstedt R Carlsson H & Fallstrom S Secretory IgA antibodies against cow's milk proteins in human milk and their possible effect in mixed feeding *Int Arch Allergy Appl Immun* 1 54 457 1977
- 10 Hjalmarson O Hanson L Å & Nilsson L Å IgA deficiency during D-penicillamine treatment *Br Med J* 1 549 1977
- 11 Immonen P Levels of serum immunoglobulins γ A γ G and γ M in the malabsorption syndrome in children *Ann Paediatr Fenn* 13 115 1967
- 12 Kuitinen J Selective IgA deficiency in blood donors *Acta Sin* 29 1 1975
- 13 Kuitinen P Duodeno-jejunal histology in malabsorption syndrome in infants *Ann Paediatr Fenn* 17 101 1966
- 14 Lawton A R Wu L Y E & Cooper M D The cellular basis of IgA deficiency in human *In* J Me stecky & A R Lawton (eds) *The Immunoglobulin A system* Plenum Press New York and London 1974 p 373
- 15 Lewkonia M M Gardner D & Doe W F IgA deficiency in one of identical twins *Br Med J* 1 311 1976
- 16 Pelkonen P Savilahti E Westerén L & Makela A L IgA deficiency in juvenile rheumatoid arthritis *Scand J Rheumatol Suppl* 8 013 1975
- 17 Ploem J S The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescence microscopy with incident light *Z Wiss Mikr* 68 1 9 1967
- 18 Rowe D S Anderson S G & Grab E A re search standard for human serum immunoglobulin IgG and IgM *Bull WHO* 47 535 1970
- 19 Rubin W Fauci A S Sleisenger M H & Jeffries G H Immunofluorescent studies in adult celiac disease *J Clin Invest* 44 475 1965
- 20 Sahr T Isokoski M Jussila J & Launiala K Lactose malabsorption in Finnish children of school age *Acta Paediatr Scand* 61 11 1971
- 21 Savilahti E Pelkonen P & Visakorpi J K IgA deficiency in children A clinical study with special reference to intestinal findings *Arch Dis Child* 46 665 1971
- 22 Savilahti E Immunoglobulin-containing cells in the intestinal mucosa and immunoglobulins in the intestinal juice in children *Clin Exp Immunol* 11 415 1977
- 23 Savilahti E IgA deficiency in children Immunoglobulin-containing cells in the intestinal mucosa immunoglobulins in secretions and serum IgA levels *Clin Exp Immunol* 13 395 1973
- 24 Seager J Jamson D I Wilson J Hayward A R & Soothill J F IgA deficiency epilepsy and phenytoin treatment *Lancet* iii 637 1975
- 25 Waldmann T A Durm M Broder S Blackman M Bleas M & Strober W Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia *Lancet* i 609 1974
- 26 Walker W A Wu M Isselbacher K J & Bloch K J Intestinal uptake of macromolecules III Studies on the mechanism by which immunization interferes with antigen uptake *J Immunol* 115 854 1975

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REFERENCES

- Aarlt J A & Tonder O Effect of antiepileptic drugs on serum and salivary IgA *Scand J Immunol* 4 391 1975
- Ammann A J & Hong R Selective IgA deficiency Presentation of 30 cases and a review of the literature *Medicine* 50 723 1971
- Andersen P & Mosekilde L Immunoglobulin levels and autoantibodies in epileptics on long term anticonvulsant therapy *Acta Med Scand* 210 69 1977
- Broom B C de la Concha E G Webster A D ■ Loewi G & Asherson G L Dichotomy between immunoglobulin synthesis by cells in gut and blood of patients with hypogammaglobulinaemia *Lancet* II 53 1975
- Brandtzaeg P Ejlertsen I & Gjeruldsen S T Immunoglobulin M Local synthesis and selective secretion in patients with immunoglobulin A deficiency *Science* 160 789 1968
- Buckley R H & Dees S C Correlation of milk precipitins with IgA deficiency *N Engl J Med* 281 465 1969
- Crabbe P A Carbonara A O & Heremans J F The normal human intestinal mucosa as a major source of plasma cells containing γ A immunoglobulin *Lab Invest* 14 35 1965
- Crabbe P A & Heremans J F Lack of gamma A immunoglobulin in serum of patients with steatorrhea *Gut* 7 119 1966
- Hanson L Å Ahlstedt S Carlsson B & Fallstrom S Secretory IgA antibodies against cow's milk proteins in human milk and their possible effect in mixed feeding *Int Arch Allergy Appl Immunol* 54 437 1977
- Hjalmarson O Hanson L Å & Nilsson L Å IgA deficiency during o-penicillamine treatment *Br Med J* 1 549 1977
- Immonen P Levels of serum immunoglobulins γ A, γ G and γ M in the malabsorption syndrome in children *Ann Paediatr Fenn* 13 115 1967
- Koivunen J Selective IgA deficiency in blood donors *Scand J Clin Lab Invest* 11 1975
- Kuitunen P Duodeno-jejunal histology in malabsorption syndrome in infants *Ann Paediatr Fenn* 17 101 1966
- Lawton A R Wu L Y E & Cooper M D The cellular basis of IgA deficiency in human *In J Me sticky & A H Lawton (eds) The Immunoglobulin A system* Plenum Press New York and London 1974 p 373
- Lewkonia M M Gaurdaer D & Doe W F IgA deficiency in one of identical twins *Br Med J* 1 311 1976
- Pelkonen P Savilahti E Westeren L & Makela A L IgA deficiency in juvenile rheumatoid arthritis *Scand J Rheumatol Suppl* 8 013 1975
- Ploem J ■ The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescence microscopy with incident light *Z Wiss Mikr* 68 179 1967
- Rowe D ■ Anderson S G & Grab B A research standard for human serum immunoglobulin IgG IgA and IgM *Bull WHO* 47 535 1970
- Rubin W Fauci A S Slesinger M H & Jeffries G H Immunofluorescent studies in adult celiac disease *J Clin Invest* 44 475 1965
- Sahr T Isokoski M Jussila J & Launiala K Lactose malabsorption in Finnish children of school age *Acta Paediatr Scand* 61 11 1971
- Savilahti E Pelkonen P & Visakorpi J K IgA deficiency in children A clinical study with special reference to intestinal findings *Arch Dis Child* 46 665 1971
- Savilahti E Immunoglobulin-containing cells in the intestinal mucosa and immunoglobulins in the intestinal juice in children *Clin Exp Immunol* 11 415 1972
- Savilahti E IgA deficiency in children Immunoglobulin-containing cells in the intestinal mucosa immunoglobulins in secretions and serum IgA levels *Clin Exp Immunol* 13 395 1973
- Seager J Jamson D I Wilson J Hayward A R & Soothill J F IgA deficiency epilepsy and phenytoin treatment *Lancet* III 632 1975
- Waldmann T A Durm M Broder S Blackman M Bleese R M & Strober W Role of suppressor T cells in pathogenesis of common variable hypogammaglobulinaemia *Lancet* I 609 1974
- Walker W A Wu M Isselbacher K J & Bloch R J Intestinal uptake of macromolecules III Studies on the mechanism by which immunization interferes with antigen uptake *J Immunol* 115 854 1975

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A PROSPECTIVE STUDY OF INDIVIDUAL COURSES OF BREAST FEEDING

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ABSTRACT Sjölin S Hofvander Y and Hillervik C (Department of Paediatrics University Hospital Uppsala Sweden) A prospective study of individual courses of breast feeding *Acta Paediatr Scand* 68 521 1979—A prospective study of the course of breast feeding was carried out in 75 randomly selected women Weekly interviews were performed from the day after delivery until the termination of breast feeding but in no case for longer than 6 months In each case a detailed analysis was made of the factors leading to transient lactation crises or to complete cessation of breast feeding A second group of 71 mothers also randomly selected was interviewed in retrospect only 6 months after delivery and served as controls Twenty four weeks after delivery 47% of the mothers in the weekly interview group were still breast feeding The corresponding figure in the control group was 38% In both groups only few mothers terminated lactation for medical reasons while about one fourth stopped for some other reason and about one half because of a combination of factors Brief case reports are presented to illustrate how varying the factors were that threatened breast feeding

KEY WORDS Breast feeding lactation crisis

In a previous retrospective study we made an attempt to analyse the causes of early termination of breast feeding (1) Some general characteristics of those mothers who breast fed for more than 8 weeks were discerned In many cases it was also possible to record a reason for the early weaning Eventually however we reached the conclusion that the direct and real cause of early weaning was not revealed by this retrospective epidemiological method We therefore decided to apply quite a different approach by following the course of breast feeding continuously and intensively in a limited number of mothers from delivery until the child was weaned or 6 months old By this means we expected to obtain more detailed and more precise information about the direct causes of termination or threatened termination of breast feeding In order to estimate the interviewer's influence on the course of breast feeding a

control group of mothers were interviewed in retrospect 6 months after delivery

MATERIAL

During the period November 1973–November 1974 78 women were selected at random at the Maternity Department of the University Hospital in Uppsala Sweden Each week the two mothers who were the last to be delivered on Monday (before midnight) were selected Only healthy mothers resident in Uppsala with healthy babies normally delivered and weighing more than 3 kg were included These mothers are henceforth referred to as the *interview group* For practical reasons the study could not be conducted continuously but had to be interrupted during part of the summer and during long holidays

The two women who were delivered immediately before those in the interview group on a Monday were selected for assignment to a *control group* with the same criteria for inclusion This group also comprised 78 women

Three mothers from the interview group refused to participate after having left the maternity ward Seven mothers in the control group moved from Uppsala and were thus excluded Thus there remained 75 mothers in the *interview group* and 71 in the *control group*

Table 1 *Duration of breast feeding*

) On discharge from the maternity ward

Time weeks	Interview group		Control group	
	Completely breast fed (%)	Completely and partially breast fed (%)	Completely breast fed (%)	Completely and partially breast fed (%)
(1)	100	97	94	99
2	89	95	93	96
4	85	92	79	87
8	77	81	69	75
12	63	79	52	66
16	51	68	39	57
20	36	60	28	45
24	17	47	15	38

Partially means that cow's milk formulas were given together with breast milk

this duration was somewhat longer in the interview group and further that extremely few babies left the maternity ward solely on a diet of breast milk substitutes

II Transient lactation crises in the interview group

During the course of lactation 46 of the 75 mothers ran into breast feeding difficulties on a total of 69 occasions (Table 2). Seventeen mothers had more than one episode of this kind. The crises could be recorded and analysed through the weekly contacts. When the mother herself contacted the interviewer she was usually in immediate need of help and advice. In most cases there were obvious and often trivial causes resulting in a decreased milk output. For example the mother was perhaps temporarily tired or the child was crying for unusually long periods for no obvious reason. Sometimes however more worrying problems either alone or as part of a chain of events caused a temporary reduction of the milk output (Table 2). It appeared and this is of fundamental importance that each individual mother reacted in her own special way to motherhood and to difficult situations. Certain mothers enjoyed their maternal role and were able to quietly endure the child's crying, night feeds, sore nipples

etc. while other mothers were tied to and constantly bothered about weight curves, grams, time schedules, the child's crying and frequent breast feeds.

The number and percentage of different reasons for transient lactation crises in the interview group are presented in Table 3. Emotional disturbances in the mother (39%) and problems concerning the child (32%) were predominant. Most of the crises occurred during the first months—52% before two months and 77% before three. By general support and simple advice it was often possible to postpone weaning for a considerable time. In 53 instances out of 69 final cessation of breast feeding was delayed by 4 weeks or more and in 25 cases by 12 weeks or more.

C Reasons for curtailed breast feeding

Table 3 shows how the reasons for curtailment of breast feeding in the interview and control groups were distributed. Reasons emanating from the interplay between the mother and the environment and rational reasons were the most common in both groups. It is striking that emotional reasons were more common in the interview group than in the retrospectively interviewed control group.

A brief review of the reasons or events

The two groups were very similar with respect to age, civil status, education and type of occupation. There was a high percentage of mothers who were studying or had completed studies at a university or college (28% in both groups). About half of the mothers were primiparas.

METHODS

The interview group

Interviews were conducted regularly starting at the maternity ward on day 1 (recruiting interview) and then continuing on day 4, whereafter home visits were made when the infant was 2 weeks, 6 weeks and 3 months old. In addition, the mothers were interviewed by telephone once weekly throughout the period of breast feeding. Furthermore, they were encouraged to contact the interviewer at once if the breast feeding was disturbed or threatened or if they felt in need of support and advice. A final interview was held after 6 months with those who were still breast feeding at that time.

During these interviews a standardized questionnaire with pre-coded as well as open-ended questions was used.¹

The interviews held at home after 2 weeks, 6 weeks and 3 months were designed to provide information not only on the child's feeding but also on the mother's physical condition, her attitude to the breast feeding and her attitude to the child and to her husband and further on the extent to which she was helped with the domestic work.

The weekly interviews by telephone included questions as to whether the mother was breast feeding wholly or partially and whether she had discontinued or intended to discontinue breast feeding. If relevant, she was asked what kind and amount of breast milk replacer she was giving. If the mother was intending to stop breast feeding or if involuntary discontinuation was imminent (hereafter referred to as a lactation crisis), a more detailed analysis was made concerning the cause, mainly with open-ended questions. Immediate home visits were often made in such cases.

During the study the interviewer thus had contact with each mother a large number of times and was able to follow any events or chain of events which threatened to terminate the breast feeding.

Particularly when the mother had decided to stop breast feeding or when a lactation crisis was imminent, attempts were made during the interviews to find out and to evaluate the real cause or motive or the starting point in a chain of events.

The control group

Contact was made with these mothers 6 months after delivery. Before that time they were not aware that they had been selected for the study. Apart from data concerning their socioeconomic situation and family background, the interview included analysis with regard to the time of weaning and reasons why the mother had started to wean the child or had stopped breast feeding completely. A standardized questionnaire with pre-coded and open-ended questions was used.

All interviews in both groups were conducted by one of the authors (C. H.). All the mothers co-operated well.

Classification of reasons for transient lactation crises and curtailed breast feeding

Successful breast feeding requires harmonious interplay between nervous and hormonal impulses within the mother, between the mother and the child and between the mother and the environment at large. Any of these relationships may be disturbed and influence the course of breast feeding. During our analyses and evaluations of the numerous reasons for lactation crises or termination of breast feeding that were revealed at the interviews, it became obvious that the best and clearest way to present these reasons was to classify them according to the scheme laid out below. In many cases there were several underlying factors, but only in a few cases was there any doubt as to which was the main reason for the lactation crisis or curtailed breast feeding. Thus, it was generally easy to decide to which group a mother should be assigned. For obvious reasons the case histories presented in Tables 2 and 4 are only brief and do not tell the whole story, but nevertheless they give essential information about the causes of threatened breast feeding in these mothers.

1 Reasons connected with the mother

(a) Medical e.g. breast complications, general illness, lactation psychosis.

(b) Rational e.g. deliberate weaning usually due to gainful employment outside the home or studies or because the mother considered that she had breast fed long enough.

(c) Emotional e.g. unhappiness, anxiety, nervousness, feeling of anguish or disgust.

2 Causes connected with the environment

(a) The child e.g. illness.

(b) The husband, siblings or other close relatives or friends' advice or pressure to wean.

(c) The Child Health Centre (CHC) e.g. advice to wean because of poor weight gain.

3 Causes connected with the interplay between the mother and her environment

Assigned to this group were cases where the course of breast feeding was influenced by environmental factors but where at the same time the mother was easily disturbed owing to various factors. Some such mothers, for instance, had poor motivation for or poor knowledge about breast feeding, had little or no domestic help and were living under stress for different reasons.

RESULTS

A Duration of breast feeding

The length of the breast feeding period in the two groups is given in Table 1. It is seen that

¹ The questionnaire used can be obtained from the authors.

Table 1 *Duration of breast feeding*

(1) On discharge from the maternity ward

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(1)	95	97	94	99
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A brief review of the reasons or events

Table 2 *Reasons for lactation crises*

	Time of crisis (weeks from birth)	Duration (weeks) of	
		Complete breast feeding	Partial breast feeding
REASONS CONNECTED WITH THE MOTHER			
<i>Medical (n=10)</i>			
Gastroenteritis vomiting Doubtful about continuing breast feeding	9	>24	-
Breasts tender Anxious	9	23	2
Breasts tender	18	20	4
Gastroenteritis Worried in case milk disappears and should she breast feed?	11	70	3
Breasts tender & inflamed Anxious—can she breast feed?	3	18	2
Breasts sore Anxious—will it affect the milk supply?	3	16	4
Rheumatoid arthritis Usually takes analgesics dares not during breast feeding Thinks milk has diminished	5	14	10
Breasts tender and sore Thinks milk has lessened Tries breast pump	4	14	10
Fever 39.5°C Should she breast feed?	7	7	7
Breasts tender	5	6	1
<i>Emotional (n=77)</i>			
Irritated & anxious about breast feeding Insufficient milk in the evening?	5	>24	-
Anxiety & apathy Often dislikes breast feeding Unable to sleep Worried about coming holiday and helping parents Milk has lessened in amount	20	20	4
Worries about how much milk the baby is getting Baby vomits after each feed	19	20	4
Depressed Sister has had a miscarriage I feel no contact with her older child Now has less milk	4	20	4
Anxiety & apathy Upset that she cannot keep up with the rest of the family Does not want to stop breast feeding entirely	13	18	6
Tired of breast feeding doesn't know why Thinks it would be nice to finish	18	18	2
Worried about having to breast feed so often Is there anything wrong with the milk?	15	18	2
Anxious about the breast feeding and whether the baby will go up in weight—baby refusing to suck Breast pump used several times a day but the milk has not increased	9	16	4
Nervous & irritated about breast feeding, and not knowing whether the baby has had enough	11	14	10
Tired of breast feeding feels very tired	10	14	10
Nervous as she thinks she may be pregnant again	10	12	1
Has stopped using the breast pump between feeds—finds it unpleasant The milk has suddenly dried up completely Very anxious	8	12	1
Very nervous disturbed by the child's dependence The child is restless wakes at night and cries often	4	12	1
Worried about the amount of milk The child has started wanting a night feed again	16	11	13
Worried about amount of milk Obsessed by weighing before and after feeds	11	11	13
Anxious The child has not gained sufficient weight	10	11	2
Tired of breast feeding wants to stop	6	11	1
Anxious about time spent on breast feeding while older child is demanding attention Would like to give the bottle occasionally	14	10	5
Depressed Tired of frequent breast feeding during the night Wants to give a bottle in the evening so that the child will sleep longer	3	10	5
Tired of breast feeding Baby fretful after every feed Has started to weigh the baby again before and after feeds	9	9	8
Finds breast feeding mentally tiring—would almost like to stop	10	9	5
Very worried about the amount of milk—the child whines despite 7 breast feeds per day The mother is anxiously checking the feeds by weighing	6	9	5
	4	9	5

Table 2. *Cont.*

	Time of crisis (weeks from birth)	Duration (weeks) of	
		Complete breast feeding	Partial breast feeding
Anxious. She does not have as much milk as she should have according to the table	6	9	3
Tired of breast feeding. Would like to give supplementary feeds	5	11	3
Dislikes breast feeding. Feels as if her breasts are "going to crack"	3	5	18
Desperate. The child has lost weight. CHC nurse has advised breast feeding every 2 h—this is too much for the mother	7	2	2
REASONS CONNECTED WITH THE ENVIRONMENT			
<i>Child (n=22)</i>			
Child has a cold and is refusing to suck. Mother worried about the child's feeds during a planned visit to friends with the family	18	>24	—
Child needs feeding every 2 h. Mother exhausted	14	>24	—
Child has a fever and cough and not sucking well. The mother is afraid her milk will dry up	1	>24	—
Child has not gained sufficient weight	7	>4	—
Child is discontented in the evening. Will not suck more than 5 times a day. Mother tired, would like to start a bottle	5	>24	—
Child discontented. Does not seem satisfied. Mother worried	4	>24	—
Child spits out the nipple. Mother tired and not sleeping	5	>24	—
Child cries. Is not satisfied. Awake at night, sleeps in the daytime	4	>24	—
Child has begun to cry 2 h after each feed. Mother tired, milk diminishing	5	>24	—
Child in hospital with fungal infection	7	2	7
Child has refused to feed all day. Mother desperate	16	18	2
Child discontented, cries. Mother depressed, believes she has no milk	11	18	2
Child refusing to suck. Mother irritated, convinced she has less milk	16	16	1
Child wanting night feeds again, cries a lot. Mother depressed, thinks her milk has diminished	8	16	1
Child cries all the afternoons despite breast feeding every 3 h	7	17	9
Child refused to suck at the maternity ward	1	11	13
Child crying, not satisfied. Mother would like to give a bottle occasionally. Baby's sister jealous	11	11	3
Child refuses to suck. Mother has started menstruating and has less milk. Wants to start weaning	10	11	2
Child still wanting night feeds. Mother tired of breast feeding	11	10	5
Child has not gained sufficient weight. CHC nurse has prescribed supplementary feeds	5	6	2
Child crying often. Mother tired, thinks that she has insufficient milk	11	5	18
Child cries after every feed. Mother anxious, would like to start with the bottle	7	2	4
<i>Her hand, siblings etc. (n=5)</i>			
The mother's mother visiting—negative to breast feeding and disturbing influence. Mother tired	4	17	7
Had to do some gardening. Exhausted, milk dried up a little	3	11	1
Fallen out with her mother in law. Milk dried up	6	7	8
Tired and disturbed by older children. Never has a chance to rest. The baby is fretful in the evenings	4	5	11
Home help has left and the mother now has 5 children to look after. Child fretful after meals	3	2	3
REASONS CONNECTED WITH INTERPLAY BETWEEN MOTHER AND ENVIRONMENT (n=9)			
Stressed and nervous after christening with many guests. Milk diminished	9	20	4
The mother is on holiday. The baby is off balance and the mother anxious	11	16	4
Mother has started menstruating. Hot summer. Mother in law on the way. Stressed. Milk drying up	13	13	11
On holiday with family. Tired and stressed. Child fretful	23	17	11
Christmas stress. Child whining. Tired of breast feeding every 15 minutes	8	6	3

Table 3 *Reasons for transient lactation crises and curtailed breast feeding (before 6 months)*

Reason connected with	Lactation crises		Curtailed breast feeding			
	Interview group		Interview group		Control group	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
The mother						
Medical	10	14	3	7	4	7
Rational	0	0	12	26	16	27
Emotional	27	39	10	22	5	8
The environment						
Child	22	32	0	0	0	0
Husband/siblings etc	5	7	0	0	2	3
Child Health Centre	0	0	1	2	2	3
The interplay between mother and environment	5	7	20	43	31	52
	69	100	46	100	60	100

that finally resulted in a complete cessation of breast feeding in each individual mother is given in Table 4.

COMMENTS

From the findings in a previous study we concluded that the true or immediate reasons for curtailed breast feeding were difficult to reveal in retrospect (1). By the retrospective method it was only possible to define in more general sociological terms the kind of mothers who tended to breast feed their infants for longer or shorter periods of time. The present investigation has clearly demonstrated that by the prospective approach with continuous and intense contact with every mother it is as a rule easy to identify the cause or causes leading to a lactation crisis or weaning.

An analysis of the brief case histories presented in Tables 2 and 4 shows above all a great variety of immediate reasons for early weaning. Sometimes several problems in combination seemed to form the causative factor and in some cases there was a chain of more or less harmless events that sooner or later resulted in a lactation crisis or weaning. Problems connected with the child obviously played a greater role as the cause of transient lactation crises than when the crises ended

in permanent termination of breast feeding (Table 3). It also seemed that emotional reasons were much more common as a cause of lactation crises in the interview group than in the control group in which the mothers were interviewed for the first time 6 months after the birth of the child. The mothers themselves seem to have difficulties in recognising or accepting in retrospect the emotional strains that may be involved in breast feeding.

A detailed study of the case histories revealed that many mothers had a tendency to exaggerate the importance of minor illnesses in themselves or in the child. Anxiety was often due to rather trivial or seemingly irrelevant incidents. If for example the child vomited a little after some meals, if the child refused to suck at a few meals or started to cry for some unknown reason, then some mothers tended to focus all their attention on the feeding and these unnecessary worries often ended up in a lactation crisis. In some cases even minor deviations from the ordinary habits of life caused an interruption of breast feeding. Visits or undue intervention by relatives and the demands placed on the mother during holidays or other celebrations seemed to be particularly harmful to breast feeding. Also the needs of other children in the family prompted some mothers to wean early. In

Table 4 *Reasons for termination of breast feeding before 6 months in the interview group*

	Duration (weeks) of	
	Complete breast feeding	Partial breast feeding thereafter
REASONS CONNECTED WITH THE MOTHER		
<i>Medical (n=3)</i>		
Retracted nipples	0	1
Neurosis	0	0
Post partum psychosis	0	0
Average	0.0	0.3
<i>Rational (n=12)</i>		
Studies	20	4
Employment	20	4
Considers she has breast fed long enough Wants to take driving lessons	20	3
Considers she has breast fed long enough Wants to attend union meetings	18	6
Employment and studies	16	4
Breast feeding terminated because of the child's age	15	11
Studies	13	7
Studies	17	7
Employment	10	4
Studies	5	18
Considers she has breast fed long enough Large family to look after	5	11
Mother of 5 with much domestic work and no home help	7	3
Average	17.8	6.5
<i>Emotional (n=10)</i>		
Feelings of restlessness and stress—not enough time to do what she likes		
Can't stand breast feeding any longer	18	2
Does not get on well with breast feeding gets tired of constraint and irregular hours Wants to be free for the summer	16	4
Cannot stand the child's total dependence	11	13
Depressed and uncomfortable with breast feeding Older child jealous	11	3
Breast feeding feels like a burden hard work one's physical strength disappears Feels disturbed by the child's total dependence	9	9
Previous depression afraid of having a relapse The child sucks the juice out of me	9	5
Finds breast feeding unpleasant slushy Wants to be free for the vacation	9	2
Hard and tiresome to breast feed Can't manage to breast feed frequently the child is underweight and is prescribed bottle feeds	6	2
Feelings of disgust for breast feeding	2	1
Dislikes breast feeding from before This child jaundiced after birth and the mother stops breast feeding immediately	1	11
Average	9.2	4.1
REASONS CONNECTED WITH THE ENVIRONMENT		
<i>Child health centre (n=1)</i>		
Wanted to breast feed but CHC persistently urged her to give supplementary feeds as the child was underweight	12	9
REASONS CONNECTED WITH THE INTERPLAY BETWEEN MOTHER AND ENVIRONMENT (n=19)		
<i>The mother felt constrained all the time Told by the CHC to wean at 4 months but did not wean until 5 months having misunderstood an information leaflet</i>		
Repeated refusal to suck The mother gets depressed and long to be back home in the country CHC encourages her to give the bottle advice followed 11 once	20	4
The mother did not want to breast feed more than 3 meals and started weaning The child refused to suck and the mother dried up	18	2
	17	4

Table 3 *Reasons for transient lactation crises and curtailed breast feeding (before 6 months)*

Reason connected with	Lactation crises Interview group		Curtailed breast feeding			
			Interview group		Control group	
	n	%	n	%	n	%
The mother						
Medical	10	14	3	7	4	7
Rational	0	0	12	26	16	27
Emotional	27	39	10	22	5	8
The environment						
Child	22	32	0	0	0	0
Husband siblings etc	5	7	0	0	2	3
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mothers also seemed to need help in their efforts to reach a sensible decision

The most impressive experience gained during this investigation was that each individual mother seemed to react in her own individual way to problems. Some were extremely sensitive and needed much support while others seemed able to cope with fairly severe strain without much ado. The differences might have been a question of disposition or constitution. To what extent a sense of security and knowledge about breast feeding played a role cannot be decided from the study.

In 1972 only four per cent of the mothers in Uppsala still nursed their babies at 24 weeks while in the present series from 1974 the corresponding figure for the control group was 38% and for the interview group 47%. The difference between the control and interview group is probably due to the more regular and intense contacts maintained by the interviewer in the latter group. The marked increase in the average duration of breast feeding over a period of a few years is certainly primarily and basically the result of an altered attitude among young women towards breast feeding. This change began slowly partly thanks to the activities of La Leche League but was speeded up quickly by the help of mass media and also by measures taken by national and local health authorities. It is

interesting to note that the baby food manufacturers have not attempted any counter activities although the sale of breast milk substitutes during the last few years had decreased markedly. On the contrary they have accepted the development with understanding willingness. However this study has shown that even if mothers in general really want to breast feed their infants this fact is no guarantee for success. Many mothers obviously need much direct support and advice to be able to complete a reasonably long lactation period.

In brief our conclusion is that breast feeding is most likely to succeed and to be continued if the mothers have learned and accepted the concept that breast feeding is superior to artificial feeding during the first months of life and if they are offered immediate and competent practical help in the event of feeding problems or other difficulties.

REFERENCE

1. Sjölin S, Hofvander Y & Hillervik C. Factors related to early termination of breast feeding. A retrospective study in Sweden. *Acta Paediatr Scand* 66: 505 1977.

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Table 4 *Cont*

	Duration (weeks) of	
	Complete breast feeding	Partial breast feeding thereafter
The child refused to suck for long period over Christmas. Pressure from grandparents and husband to give breast milk replacer. When home again the mother tries to breast feed wholly but the child refuses and continues to prefer the bottle and the mother gives up	16	1
The mother finds breast feeding tiresome when there is not enough milk. Does not want to feed frequently	12	6
The mother young, nervous and stressed about poor relations with her husband. The child whining, the mother feeds frequently without result	12	1
The child starts to wake up at night. The mother is menstruating and has less milk. Feels tied to the question of milk amount	11	2
The child cries day and night. The mother anxious, breast feeds frequently as the child was found to be underweight at CHC. Advice from CHC to weigh breast feeds and to give the bottle or stop breast feeding. The mother tired of the frequent breast feeding and of the other children and stops breast feeding	11	1
The child still wants night feeds at 3 months. Older siblings demanding attention	10	5
The mother tired, has little help, child crying at night, long period of mixed feeding. The child gets pneumonia and is admitted to hospital. The mother doesn't want to go there for breast feeding	9	8
Whining child. The mother has breast fed every 3 h for 2 days. Husband dislikes both the breast feeding and the survey. The mother despaired and introduces the bottle	7	8
The mother unwell. CHC finds the child underweight. The mother has tried frequent breast feeding without result. Convinced she is not able to breast feed	7	7
The mother irritated about breast feeding, has a cold, feels tired, too much Christmas cleaning. Breast fed frequently for 6 days without result. The child quieter after start of bottle feeding	6	3
The mother sensitive and nervous before Christmas and the gathering of many relatives. Dries up and the child is whining. Pressure from various people to give the bottle	4	1
The child not content despite 6-9 feeds a day, 1 hour each time. The mother tired and dries up suddenly	3	2
The child refuses to suck temporarily, the mother gives up and does not want to try again (feels averse to the child)	3	0
Whining child, mother anxious about the milk amount, wants to participate in the local spring festival and starts the child on the bottle	2	4
The mother wants to breast feed but cannot relax, is nervous, tense and anxious about the milk amount. Siblings jealous when the baby is fed	2	2
The child cries, 4 persons living in the same room. The mother dries up	0	6
Whining child, demanding siblings, no domestic help, no time to rest	0	6
Average	9.0	3.6

many instances the mother did not feel at ease with breast feeding. Some of them felt too tied while others were unable to stand the child's total dependence. Some mothers found breast feeding unpleasant. In a few cases an insufficiently understanding husband was obviously a major reason for early weaning.

In many cases weaning could be avoided by simple advice and general support. This

was true especially when the mother was unduly worried or when the immediate problem could be easily eliminated.

Sometimes all attempts to help failed completely. This happened in particular with mothers who for some reason did not really want to go on with breast feeding. In other instances early weaning seemed to be unavoidable for quite rational reasons or owing to a pressing situation in the family. These

SOCIAL BACKGROUND AND LIFE EVENTS OF CHILDREN ADMITTED TO A PAEDIATRIC DEPARTMENT

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ABSTRACT Aagaard J (Department of Paediatrics Randers Centralsygehus Denmark and Institute of Social Medicine University of Aarhus Denmark) Social background and life events of children admitted to a paediatric department. *Acta Paediatr Scand* 68 531 1979. — During a period of one year a questionnaire was filled in by parents of children who were more than one year old and admitted to the paediatric department in Randers, Denmark. The questionnaire was especially concerned with factors describing the social background of the family and with selected life events. Family background variables such as low level of parents' education and low income were found to be associated with psychosomatic and psychic diseases of the children. No associations between these negative family background variables and respiratory tract infections were found. The data show a strikingly high frequency of selected life events. Noticeable intercorrelations were found between social factors, life events and diagnostic groups. In the light of this it was not considered meaningful to analyze life events independently of the family background variables. A combined measurement for family background variables and selected life events was strongly associated with psychosomatic and psychic diseases. The additional information due to life events over the information due to family background variables was pronounced. It was remarkable that stressful life events also were frequent among children with some somatic diseases. The inference of these results is a plea for application of analysis of individual social history to more hospitalized children. The results might further have implications for preventive work with respect to the changes which seem to have taken place in important social conditions.

KEY WORDS Social paediatrics, life events, hospital admission.

Many investigations have dealt with social background factors for children admitted to hospital (11-14). The reported investigations are, however, criticizable from a methodological point of view. One usually notes especially that only more stable social variables are taken into account, and there is no attempt to evaluate the relative significance of the elements in the social history of the child.

It has been shown in some investigations that many children in a paediatric department have prior psychic deviating development and/or emotional disturbances (9-13). Thus Apley & McKeith (1) have emphasized that various organic symptoms often have a psychic background.

In addition to this aspect there seems in some cases to be a connection between earlier

diseases deviating development and later symptoms in pedagogic, social or health areas (3). It must, however, be stressed that information dealing with prediction of later illness or impaired development from essential elements in the social history is only available for certain diagnoses (10-12).

Purpose and definitions

In this part of the investigation the purpose was to estimate the relative significance of social background factors and life events for certain diagnostic groups of children admitted to the department.

By *social factors* is meant relatively stable aspects of the parents' situation such as level of education, income, social status and residence.

Table 1 *Diagnostic group by the parents' education*

Parents' education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
<i>Father's school education</i>						
≤9 years	71	32	44	135	237 (65.4)	$\chi^2 = 71.0$ $df = 3$
>9 years	19	10	7	54	85 (73.9)	
Not stated/not relevant	7	5	9	17	38 (10.7)	$p < 0.001$
Total	47 (13.3)	47 (13.3)	55 (15.5)	706 (58.0)	355 (100.0)	
<i>Father's occupational education</i>						
≤1 year	6	10	72	65	103 (79.0)	$\chi^2 = 17.7$ $df = 3$
>1 year	35	3	74	174	215 (60.6)	
Not stated/not relevant	6	5	9	17	37 (10.4)	$p < 0.01$
Total	47 (13.3)	47 (13.3)	55 (15.5)	706 (58.0)	355 (100.0)	
<i>Mother's school education</i>						
≤9 years	27	27	51	153	258 (72.7)	$\chi^2 = 71.7$ $df = 3$
>9 years	70	19	4	52	95 (76.8)	
Not stated	0	1	0	1	2 (0.6)	$p < 0.001$
Total	47 (13.3)	47 (13.3)	55 (15.5)	706 (58.0)	355 (100.0)	
<i>Mother's occupational education</i>						
≤1 year	70	4	39	111	204 (57.5)	$\chi^2 = 10.1$ $df = 3$
>1 year	6	77	14	8	144 (40.6)	
Not stated	1	1	~	3	7 (1.9)	$p = 0.05$
Total	47 (13.3)	47 (13.3)	55 (15.5)	706 (58.0)	355 (100.0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents' school education and significant differences with respect to the length of the parents' occupational education.

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education. This corresponds to the condition of the area population.

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training while infections of the respiratory tract contained predominance of parents with a higher education.

Table 2 shows diagnostic groups by selected family background variables. The dividing value for taxable income at 60000 D kr corre-

sponds to the average taxable income for an unskilled worker. As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups. This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income.

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room. Neither parameter distinguished significantly between diagnostic groups.

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available.

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10.1% and of parents 22.8%.

By life events in merit social events such as divorce unemployment and changes in inter personal relationships

METHOD

The investigation consisted of questionnaires filled in by parents of children admitted to the paediatric department or admitted to the surgical department for herniotomy. The hospital receives children from a geographically relatively well defined area. There is no paediatric out patients clinic in the area. There is no social selection for admission to hospital.

Prior to this investigation a pilot study was undertaken and published (15, 16). The questionnaire dealt with relatively stable social background variables and changes in the circumstances of life which had occurred during the year prior to admission. A slightly modified edition of Coddington's life event list was used (4, 5). A critical evaluation of Coddington's life event/life change unit (LCU) method has been published earlier (17).

The questionnaires were distributed by the head nurse of the department. The procedure followed was in accordance with the demands of the Helsinki II Declaration (8).

For the non response group selected social variables were obtained from the medical record. For all patients diagnosis admission type and duration of stay were recorded.

The validity of each diagnosis was confirmed by the chief physician of the department. The main diagnoses were aggregated in diagnostic groups. The group respiratory tract infections consisted of 38 preschool children (age 1-6) of whom 25 had lower and 13 had upper respiratory tract infections and 9 elementary school children (age 7-14) of whom 7 had lower and 2 had upper respiratory tract infections. The group of infections outside the respiratory tract consisted of 31 preschool children of whom 8 had gastroenteritis and 11 had urinary tract infections and 16 elementary school children of whom 5 had infections of the urinary tract and 5 had meningitis/sepsis. The group psychosomatic and psychic diseases consisted of 70 preschool children of whom 7 had enuresis and 6 encopresis and 35 elementary school children of whom 13 had abdominal colic 12 had enuresis and 5 had infantile neurosis. The group other diseases consisted of 139 preschool children of whom 5 had a diagnosis of accidental drug poisoning 12 had other forms of poisoning 12 had various symptoms from the gastrointestinal tract 30 had febrile convulsions and 9 had epilepsy and 67 elementary school children of whom 9 had epilepsy 10 had asthma bronchiale 5 were admitted for herniotomy and 5 had diabetes mellitus.

The reliability of the information given in the questionnaire was judged by comparing this information with the analogous data from the medical record. Social background variables showed a great deal of agreement between the record data and questionnaire data. The reliability of the information concerning life events could not be estimated as the majority of records was incomplete on this point.

Statistical procedures

The material was processed at the Regional EDP Center University of Aarhus (RECAU). The program for appropriate scoring was written by Professor John Ipsen. A further description of the statistical procedures is published elsewhere (17).

By a profile of a diagnostic group is meant the array of normalized mean for the independent variables. The variables used in this investigation were all dichotomized. The normalized means are random variates of a normal distribution with mean equal to zero and unit standard error.

Combined variables were computed. These variables were the sum of selected profile elements multiplied by the appropriate coefficient for the individual element. The appropriate coefficients were calculated by a process which corrected for intercorrelations between the independent variables. The combined measurements made in this connection be understood as a weighted index which takes negative social factors and selected life events into account.

MATERIAL

The patient group consisted of all children over one year of age who were admitted in the period Oct. 1st 1971 - Sept. 30th 1977.

Excluded from participation in the investigation were 1) Children of foreign language speaking parents 2) institutionalized children 3) Children transferred to other hospital within the first day after admission 4) Children who were readmitted during the period of investigation 5) Children who stayed in hospital less than 48 hours 6) Children who had participated in the pilot study (16). In the period of investigation 482 children were admitted and 22 (4.6%) were excluded.

The occupational group distribution of the fathers of the children corresponded to that of men in the same age group in the population of the area (6).

Five (1.1%) of the children's parents did not want to participate in the investigation. 455 questionnaires were distributed 375 (82.4%) of these were returned 14 (3.7%) of the returned questionnaires were incompletely filled in. Family background variables did not differ significantly between response and non response groups.

Of the response group's 361 patients 187 were boys and 174 were girls. The average age was 5.3 years (SD 3.8). The average stay in hospital was 6.9 days (SD 4.6). 22% (63.2%) of the children were at preschool age 127 (35.2%) were at elementary school age and 6 (1.6%) were older. As the significance of life events among other things is age dependent (5) the children over 14 years of age were excluded from the following analysis. The reduced study population then consisted of 355 patients.

RESULTS

Diagnostic groups by social factors

The children's main diagnoses at time of discharge from hospital are in Tables 1 and 2 reduced to three groups and a remainder group.

Table 1 *Diagnostic group by the parents' education*

Parents' education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
<i>Father's school education</i>						
≤9 years	71	37	44	135	237 (65.4)	$\chi^2=71.0$ $df=3$
>9 years	19	10	2	54	85 (23.9)	
Not stated/not relevant	7	5	9	17	38 (10.7)	$p<0.001$
Total	47 (13.7)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Father's occupational education</i>						
≤1 year	6	10	77	65	103 (29.0)	$\chi^2=17.7$ $df=3$
>1 year	35	37	4	124	215 (60.6)	
Not stated/not relevant	6	5	9	17	37 (10.4)	$p<0.01$
Total	47 (13.2)	47 (13.1)	55 (15.5)	106 (58.0)	355 (100.0)	
<i>Mother's school education</i>						
≤9 years	7	27	51	153	238 (77.7)	$\chi^2=21.7$ $df=3$
>9 years	70	19	4	57	95 (26.8)	
Not stated	0	1	0	1	2 (0.6)	$p<0.001$
Total	47 (13.7)	47 (13.7)	55 (15.5)	106 (58.0)	355 (100.0)	
<i>Mother's occupational education</i>						
≤1 year	70	74	39	171	204 (57.5)	$\chi^2=10.1$ $df=3$
>1 year	76	7	14	82	144 (40.6)	
Not stated	1	1	2	3	7 (1.9)	$p<0.05$
Total	47 (13.2)	47 (13.2)	55 (15.5)	106 (58.0)	355 (100.0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents' school education and significant differences with respect to the length of the parents' occupational education.

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education. This corresponds to the condition of the area population.

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training while infections of the respiratory tract contained predominance of parents with a higher education.

Table 2 shows diagnostic groups by selected family background variables. The dividing value for taxable income at 60 000 D kr corre-

sponds to the average taxable income for an unskilled worker. As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups. This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income.

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room. Neither parameter distinguished significantly between diagnostic groups.

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available.

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10.1% and of parents 22.8%.

By life events is meant social events such as divorce, unemployment and changes in interpersonal relationships.

METHOD

The investigation consisted of questionnaires filled in by parents of children admitted to the paediatric department or admitted to the surgical department for herniotomy. The hospital receives children from a geographically relatively well defined area. There is no paediatric out-patients clinic in the area. There is no social selection for admission to hospital.

Prior to this investigation a pilot study was undertaken and published (15, 16). The questionnaire dealt with relatively stable social background variables and changes in the circumstances of life which had occurred during the year prior to admission. A slightly modified edition of Coddington's life event list was used (4, 3). A critical evaluation of Coddington's life event/life change unit (LCU) method has been published earlier (17).

The questionnaires were distributed by the head nurse of the department. The procedure followed was in accordance with the demands of the Helsinki II Declaration (18).

For the non-response group selected social variables were obtained from the medical record. For all patients diagnosis, admission type and duration of stay were recorded.

The validity of each diagnosis was confirmed by the chief physician of the department. The main diagnoses were aggregated in diagnostic groups. The group respiratory tract infections consisted of 38 preschool children (age 1-6) of whom 25 had lower and 13 had upper respiratory tract infections and 9 elementary school children (age 7-14) of whom 7 had lower and 2 had upper respiratory tract infections. The group of infections outside the respiratory tract consisted of 31 preschool children of whom 8 had gastroenteritis and 11 had urinary tract infections and 16 elementary school children of whom 5 had infections of the urinary tract and 9 had meningitis/sepsis. The group psychosomatic and psychic diseases consisted of 20 preschool children of whom 7 had enuresis and 13 enco encopresis and 35 elementary school children of whom 13 had abdominal colic, 12 had enuresis and 11 had infantile neurosis. The group other diseases consisted of 139 preschool children of whom 5 had a diagnosis of accidental drug poisoning, 12 had other forms of poisoning, 12 had various symptoms from the gastrointestinal tract, 30 had febrile convulsions and 9 had epilepsy and 67 elementary school children of whom 9 had epilepsy, 10 had asthma bronchiale, 5 were admitted for herniotomy and 5 had diabetes mellitus.

The reliability of the information given in the questionnaire was judged by comparing this information with the analogous data from the medical record. Social background variables showed a great deal of agreement between the record data and questionnaire data. The reliability of the information concerning life events could not be estimated as the majority of records was incomplete on this point.

Statistical procedures

The material was processed at the Regional EDP-Center University of Aarhus (RECAU). The program for appropriate scoring was written by Professor John Ipsen. A further description of the statistical procedures is published elsewhere (17).

By a profile of a diagnostic group is meant the array of normalized mean for the independent variables. The variables used in this investigation were all dichotomized. The normalized means are random variates of a normal distribution with mean equal to zero and unit standard error.

Combined variables were computed. These variables were the sum of selected profile elements multiplied by the appropriate coefficient for the individual element. The appropriate coefficients were calculated by a process which corrected for intercorrelations between the independent variables. The combined measurements might in this connection be understood as a weighted index which takes negative social factors and selected life events into account.

MATERIAL

The patient group consisted of all children over one year of age who were admitted in the period Oct. 1st 1965 Sept. 30th 1977.

Excluded from participation in the investigation were 1) Children of foreign language speaking parents, 2) Institutionalized children, 3) Children transferred to another hospital within the first day after admission, 4) Children who were readmitted during the period of investigation, 5) Children who stayed in hospital less than 24 hours, 6) Children who had participated in the pilot study (16). In the period of investigation 482 children were admitted and 22 (4.6%) were excluded.

The occupational group distribution of the fathers of the children corresponded to that of men in the same age group in the population of the area (6).

Five (1.1%) of the children's parents did not want to participate in the investigation. 455 questionnaires were distributed, 375 (82.4%) of these were returned, 14 (3.7%) of the returned questionnaires were incompletely filled in. Family background variables did not differ significantly between response and non-response groups.

Of the response group's 361 patients, 197 were boys and 174 were girls. The average age was 5.3 years ($SD = 3.8$). The average stay in hospital was 6.9 days ($SD = 4.6$). 27% (63.2%) of the children were at preschool age, 127 (35.2%) were at elementary school age and 6 (1.6%) were older. As the significance of life events among other things is age dependent (5), the children over 14 years of age were excluded from the following analysis. The reduced study population then consisted of 355 patients.

RESULTS

Diagnostic groups by social factors

The children's main diagnoses at time of discharge from hospital are in Tables 1 and 2 reduced to show the distribution of the main diagnostic groups.

Table 1 Diagnostic group by the parents' education

Parents' education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
<i>Father's school education</i>						
≤9 years	21	3	44	135	233 (65.4)	$\chi^2=21.0$ $df=3$
>9 years	19	10		54	83 (23.9)	
Not stated/not relevant	7	5	9	17	38 (10.7)	$p<0.001$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Father's occupational education</i>						
≤1 year	6	10	22	65	103 (29.0)	$\chi^2=12.7$ $df=3$
>1 year	35	37	4	174	215 (60.6)	
Not stated/not relevant	11	5	9	17	37 (10.4)	$p<0.01$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Mother's school education</i>						
≤9 years	27	27	51	153	258 (72.7)	$\chi^2=21.7$ $df=3$
>9 years	20	19	4	52	95 (26.8)	
Not stated	0	1	0	1	2 (0.6)	$p<0.001$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Mother's occupational education</i>						
≤1 year	20	24	39	121	204 (57.5)	$\chi^2=10.1$ $df=3$
>1 year	26	2	14	8	144 (40.6)	
Not stated	1	1	2	3	7 (1.9)	$p<0.05$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents' school education and significant differences with respect to the length of the parents' occupational education.

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education. This corresponds to the condition of the area population.

In the four diagnostic groups psychosomatic and psychic diseases were marked by an excess of parents with short education and training while infections of the respiratory tract contained predominance of parents with a higher education.

Table 2 shows diagnostic groups by selected family background variables. The dividing value for taxable income at 60 000 D kr corre-

sponds to the average taxable income for an unskilled worker. As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups. This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income.

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room. Neither parameter distinguished significantly between diagnostic groups.

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available.

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10.1% and of parents 22.8%.

By *life events* is meant social events such as divorce, unemployment and changes in interpersonal relationships.

METHOD

The investigation consisted of questionnaires filled in by parents of children admitted to the paediatric department or admitted to the surgical department for herniotomy. The hospital receives children from a geographically relatively well-defined area. There is no paediatric outpatient clinic in the area. There is no social selection for admission to hospital.

Prior to this investigation a pilot study was undertaken and published (15, 16). The questionnaire dealt with relatively stable social background variables and changes in the circumstances of life which had occurred during the year prior to admission. A slightly modified edition of Coddington's life event list was used (4, 9). A critical evaluation of Coddington's life event/life change unit (LCU) method has been published earlier (17).

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For the non-response group selected social variables were obtained from the medical record. For all patients diagnosis, admission type and duration of stay were recorded.

The validity of each diagnosis was confirmed by the chief physician of the department. The main diagnoses were aggregated in diagnostic groups. The group respiratory tract infections consisted of 38 preschool children (age 1-6) of whom 25 had lower and 13 had upper respiratory tract infections and 9 elementary school children (age 7-14) of whom 7 had lower and 2 had upper respiratory tract infections. The group of infections outside the respiratory tract consisted of 31 preschool children of whom 8 had gastroenteritis and 11 had urinary tract infections and 16 elementary school children of whom 5 had infections of the urinary tract and 5 had meningitis/sepsis. The group psychosomatic and psychic diseases consisted of 20 preschool children of whom 7 had enuresis and 11 encopresis and 35 elementary school children of whom 13 had abdominal colic, 12 had enuresis and 6 had infantile neurosis. The group other diseases consisted of 139 preschool children of whom 5 had a diagnosis of accidental drug poisoning, 12 had other forms of poisoning, 12 had various symptoms from the gastrointestinal tract, 30 had febrile convulsions and 9 had epilepsy and 67 elementary school children of whom 9 had epilepsy, 10 had asthma bronchiale, 5 were admitted for herniotomy and 5 had diabetes mellitus.

The reliability of the information given in the questionnaire was judged by comparing this information with the analogous data from the medical record. Social background variables showed a great deal of agreement between the record data and questionnaire data. The reliability of the information concerning life events could not be estimated as the majority of records was incomplete on this point.

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Combined variables were computed. These variables were the sum of selected profile elements multiplied by the appropriate coefficient for the individual element. The appropriate coefficients were calculated by a process which corrected for intercorrelations between the independent variables. The combined measurements might in this connection be understood as a weighted index which takes negative social factors and selected life events into account.

MATERIAL

The patient group consisted of all children over one year of age who were admitted in the period Oct. 1st 1976-Sept. 30th 1977.

Excluded from participation in the investigation were 1) Children of foreign language speaking parents, 2) Institutionalized children, 3) Children transferred to another hospital within the first day after admission, 4) Children who were readmitted during the period of investigation, 5) Children who stayed in hospital less than 24 hours, 6) Children who had participated in the pilot study (16). In the period of investigation 482 children were admitted and 22 (4.6%) were excluded.

The occupational group distribution of the fathers of the children corresponded to that of men in the same age group in the population of the area (6).

Five (1.1%) of the children's parents did not want to participate in the investigation. 455 questionnaires were distributed, 375 (82.4%) of these were returned, 14 (3.7%) of the returned questionnaires were incompletely filled in. Family background variables did not differ significantly between response and non-response groups.

Of the response group's 361 patients, 187 were boys and 174 were girls. The average age was 5.3 years ($SD = 3.8$). The average stay in hospital was 6.9 days ($SD = 4.6$). 228 (63.2%) of the children were at preschool age, 127 (35.2%) were at elementary school age and 6 (1.6%) were older. As the significance of life events among other things is age dependent (5), the children over 14 years of age were excluded from the following analysis. The reduced study population then consisted of 355 patients.

RESULTS

Diagnostic groups by social factors

The children's main diagnoses at time of discharge from hospital are in Tables 1 and 2 reduced to three groups and a remainder group.

Table 1 Diagnostic group by the parents' education

Parents' education	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
<i>Father's school education</i>						
≤9 years	71	37	44	135	237 (65.4)	$\chi^2=71.0$ $df=3$
>9 years	19	10	7	54	85 (23.9)	
Not stated/not relevant	7	5	9	17	38 (10.7)	$p<0.001$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Father's occupational education</i>						
≤1 year	6	10	22	65	103 (29.0)	$\chi^2=12.7$ $df=3$
>1 year	35	37	74	174	215 (60.6)	
Not stated/not relevant	11	5	9	17	37 (10.4)	$p<0.01$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Mother's school education</i>						
≤9 years	27	77	51	153	258 (72.7)	$\chi^2=21.7$ $df=3$
>9 years	0	19	4	57	95 (26.8)	
Not stated	0	1	0	1	7 (0.6)	$p<0.001$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Mother's occupational education</i>						
≤1 year	20	4	39	171	204 (57.5)	$\chi^2=10.1$ $df=3$
>1 year	6	27	14	87	144 (40.6)	
Not stated	1	1	7	3	7 (1.9)	$p<0.05$
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	

As shown in Table 1 highly significant differences were found between diagnostic groups with respect to length of the parents' school education and significant differences with respect to the length of the parents' occupational education.

The length of school education was uniform for men and women whereas a greater part of men than women had a longer occupational education. This corresponds to the condition of the area population.

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Table 2 shows diagnostic groups by selected family background variables. The dividing value for taxable income at 60 000 Dkr corre-

sponds to the average taxable income for an unskilled worker. As shown in Table 2 income is the only variable which distinguishes significantly between diagnostic groups. This association was characterized in the fact that children with psychosomatic and psychic diseases more frequently had parents with a lower income.

Place of residence was judged partly by number of rooms and partly by overcrowding defined as more than one person per room. Neither parameter distinguished significantly between diagnostic groups.

Frequencies of life events

An estimate of the frequency of life events among Danish children is not yet available.

Among preschool children the percentage frequencies of hospitalization of brother or sister were 10.1% and of parents 22.8%.

Table 2 *Diagnostic group by the parents' marital status, income and number of rooms in residence*

Parents' marital status, income and residence	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
<i>Marital status</i>						
Not married	8	4	9	16	37 (10.4)	$\chi^2=5.9$ $df=3$ $p>0.05$
Married	39	43	46	188	316 (89.0)	
Not stated	0	0	0	2	2 (0.6)	
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Income</i>						
≤60 000 D kr	10	13	28	49	100 (28.2)	$\chi^2=17.9$ $df=3$ $p<0.001$
>60 000 D kr	36	33	26	153	248 (69.9)	
Not stated	1	1	1	4	7 (1.9)	
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Residence</i>						
≤3 rooms	13	9	10	45	77 (21.7)	$\chi^2=1.6$ $df=3$ $p>0.05$
>3 rooms	34	38	45	161	278 (78.3)	
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	

Among elementary school children the analogous percentages were 15.7 and 16.5. The reasons for hospitalization of relatives were not explored.

Among the preschool children five (2.2%) had experienced death in the near family. Among elementary school children only one had lost a parent by death.

Many of those life events which were related to the parents' occupation also had a relatively high frequency. Among wage earners with preschool children 14.9% of the fathers and 19.5% of the mothers had been unemployed in the year prior to admission of the child. For 4–8% of the children there was information concerning the parents' increased absence from home due to work. Remarkably many mothers of preschool children (15.5%) had started working during the year prior to admission. Changes in the parents' financial status were as frequent as 30% among the parents of preschool children and 20% of the parents of elementary school children stated change in financial status.

6.6% of preschool children and 3.9% of elementary school children had experienced di-

vorce or separation of parents during the year prior to admission. 17.7% of the children had or had had a single supporter. Remarkably high frequencies of increased interpersonal conflicts were found: 12% reported increase in number of arguments between parents. Among preschool children the percentage of increase in number of arguments with parents or with peers was 5; the analogous percentages for elementary school children were 14.2 and 26.5.

Correlations between selected social factors, life events and diagnostic groups

As expected there were positive intercorrelations between the parents' school education, occupational education and income. Lower income was among other things correlated to increased conflicts with and between parents and to increased conflict with peers. There were also positive correlations between lower income and broken home, hospitalization of parents and changes in parents' financial status. Unemployment of fathers was positively correlated with broken home and lower in-

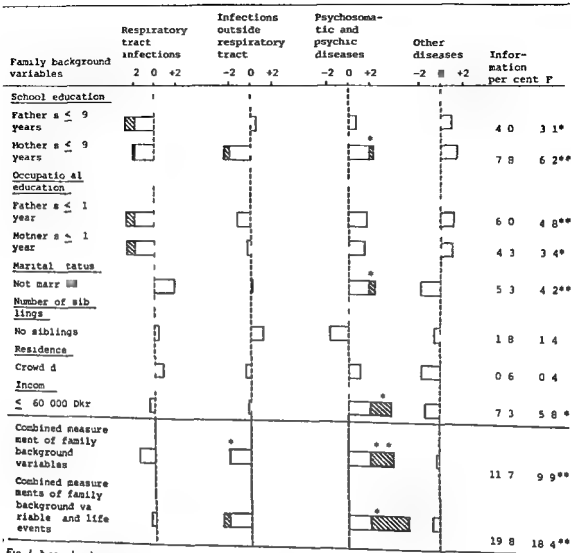


Fig 1 Normalized means for single and combined measurements of family background variables and life events for preschool children

** Significant on 1% level

* Significant on 5% level

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come and mothers unemployment was positively correlated to fathers unemployment

It is of special interest that psychosomatic diseases are positively correlated to low level of education and broken home and to events as hospitalization of siblings and increase in number of arguments with peers. The psychosomatic diseases were negatively correlated with only child. Consideration of correlation matrix might then suggest that an analysis necessarily must take into account

the intercorrelations between social factors and life events

Combined measurements of social factors and life events by diagnostic groups

Preschool children's diagnostic groups are profiled in Fig 1 by selected family background variables. The group of respiratory tract infections were characterized by higher level of education of the parents but the other parameters did not deviate significantly. The

Table 2 Diagnostic group by the parents marital status income and number of rooms in residence

Parents marital status income and residence	Respiratory tract infections	Infections outside respiratory tracts	Psychosomatic and psychic diseases	Other diseases	Total number per cent	χ^2 test (corrected)
<i>Marital status</i>						
Not married	8	4	9	16	37 (10.4)	$\chi^2=5.9$ $df=3$ $p>0.05$
Married	39	43	46	189	316 (89.0)	
Not stated	0	0	0	2	2 (0.6)	
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>60 000 D kr	36	33	26	153	248 (69.9)	
Not stated	1	1	1	4	7 (1.9)	
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	
<i>Residence</i>						
≤3 rooms	13	9	11	45	77 (21.7)	$\chi^2=1.6$ $df=1$ $p>0.05$
>3 rooms	34	38	45	161	278 (78.3)	
Total	47 (13.2)	47 (13.2)	55 (15.5)	206 (58.0)	355 (100.0)	

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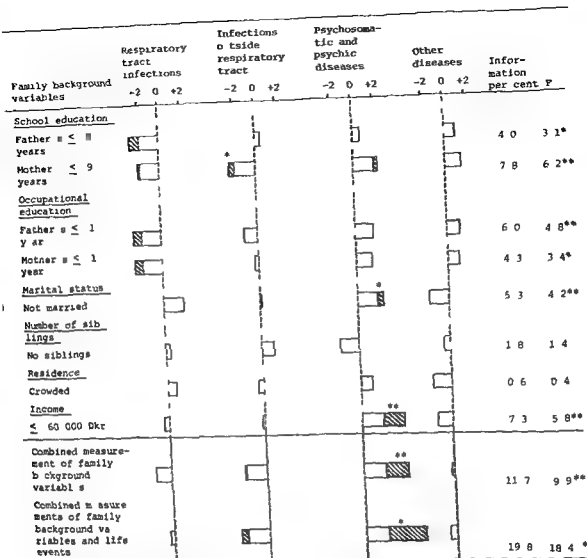


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only yield moderate information in this age group. By combining the measurements for family background variables it was found that the tendency was the same as in the group of preschool children but not so pronounced. It is essential to note however that life events did yield significant additional information and that this was related to a further significant deviation for the group of children with psychosomatic and psychic diseases.

It is now natural to ask how the selected life events are distributed on the diagnoses in the two age groups.

Preschool children's diagnostic groups are profiled in Fig. 2 by selected life events. The individual life events showed no clear tendency but there were significantly more children with respiratory tract infections who had had increased number of arguments with parents. The combined measurement for life events revealed high values among respiratory tract infections as well as psychosomatic and psychic diseases most pronounced for the latter diagnostic group.

Elementary school children's diagnostic groups were profiled in the same way by selected life events. More life events show significant deviations. Especially conflict with peers and hospitalization of siblings should be emphasized. The maximal information due to life events was significantly greater in this age group than among preschool children. The combined measurement for life events shows strong positive significant deviation in the group of psychosomatic and psychic diseases.

DISCUSSION

The investigation has a relatively large non-response group. However, there was no significant difference between composition of the response group and the non-response group.

The questions which describe the social background were relatively simple and a further simplification occurred during data processing. For those reasons such a design can

only point to statistical associations between the independent variables but beyond this a statistical estimation of the relative weight of these is possible. In other words, one cannot demonstrate causal relations but only associations with possible etiological determinants.

The classification into diagnostic groups is relatively coarse but nevertheless there was a great deal of clinical homogeneity within groups which makes meaningful explanations possible. The remainder group did not deviate significantly in any respect.

It was characteristic that the selected social factors had greatest significance among preschool children but the diagnostic groups for elementary school children tended towards the same type of profile.

That negative social factors such as low level of education and relatively low income were frequent among children with psychosomatic and/or psychic diseases and that the opposite was the case within the group of respiratory tract infections is an observation which is contrary to earlier investigations in social medicine (2).

This suggests that the significance of social factors has changed in recent time. As an example residence might be mentioned. Nearly 3/4 of the children lived in residences with more than 3 rooms and only 1/10 lived in a crowded residence. Neither number of rooms nor crowded residence seemed to be of significance in differing between groups. This has naturally something to do with these essential improvements in the technical and hygienic condition of housing which have taken place during the last generation. That does not mean that a poor residence cannot be a health risk but at present relatively few children grow up in unhealthy residences.

The absolute frequency of selected life events were relatively high but only for a few of the selected life events was there a significant association with diagnostic groups. The combined measurement for life events however showed a positive significant deviation

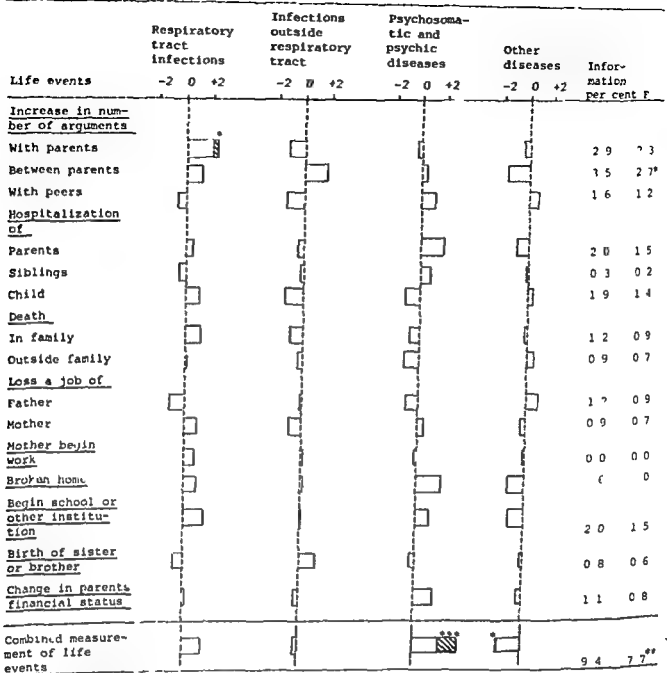


Fig. 2 Normalized means for single and combined measurements of life events for preschool children

***Significant on 1 o/oo level

** Significant on 1% level

* Significant on 5% level

group of psychosomatic and psychic diseases was characterized by low level of education not married and low income

The selected family background variables were employed to create a combined measurement which took into account the correlations between the independent variables. As shown in Fig. 1 the group of psychosomatic and psychic diseases deviated significantly

A new combined measurement including family background variables and life events increased the information a great deal. The additional information was essentially due to the further significant deviation of the group of psychosomatic and psychic diseases.

Elementary school children's diagnostic groups were profiled in the same way. It is characteristic that the individual social factors

only yield moderate information in this age group. By combining the measurements for family background variables it was found that the tendency was the same as in the group of preschool children but not so pronounced. It is essential to note however that life events did yield significant additional information and that this was related to a further significant deviation for the group of children with psychosomatic and psychic diseases.

It is now natural to ask how the selected life events are distributed on the diagnoses in the two age groups.

Preschool children's diagnostic groups are profiled in Fig. 2 by selected life events. The individual life events showed no clear tendency but there were significantly more children with respiratory tract infections who had had increased number of arguments with parents. The combined measurement for life events revealed high values among respiratory tract infections as well as psychosomatic and psychic diseases most pronounced for the latter diagnostic group.

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That negative social factors such as low level of education and relatively low income were frequent among children with psychosomatic and/or psychic diseases and that the opposite was the case within the group of respiratory tract infections is an observation which is contrary to earlier investigations in social medicine (2).

This suggests that the significance of social factors has changed in recent time. As an example residence might be mentioned. Nearly 3/4 of the children lived in residences with more than 3 rooms and only 1/10 lived in a crowded residence. Neither number of rooms nor crowded residence seemed to be of significance in differing between groups. This has naturally something to do with these essential improvements in the technical and hygienic condition of housing which have taken place during the last generation. That does not mean that a poor residence cannot be a health risk but at present relatively few children grow up in unhealthy residences.

The absolute frequency of selected life events were relatively high but only for a few of the selected life events was there a significant association with diagnostic groups. The combined measurement for life events however showed a positive significant deviation

for the group of psychosomatic and psychic diseases

It was remarkable that there was also a tendency towards large numbers of life events among preschool children who had a respiratory tract infection as main diagnosis. These children were nearly all admitted as emergencies.

However, this investigation cannot clarify whether large numbers of life events had true etiologic significance or only influenced the indication for admission to hospital.

The combined measurement was computed on the background of the intercorrelations by applying the method of appropriate scoring. The applied method differs from life event/life change unit (LCU) procedure where pre-assigned life event scores usually are applied.

With a few exceptions (7) life event/LCU research did not take into account the possible intercorrelations between life events and social factors. Noticeable correlations between social factors and life events were found in this study. On this basis it can hardly be doubted that life events should not be considered alone, but must be evaluated in connection with the family background variables.

It was not contrary to expectation that the combined measurement for family background variables and life events were strongly associated with psychosomatic and psychic diseases, but it was remarkable that the additional information from life events appeared so strongly. The results suggest that stressful life events in combination with negative social factors increases the risk of developing a psychosomatic or psychic disease of such a severity that the child has to be admitted to hospital.

Consequences of the results

The new aspect to which this study has contributed is to clarify the relative weight of family background variables and life events for various disease categories.

As stressful life events also frequently occur prior to admission of children with some somatic diseases, there is a consequent plea

for a detailed recording of the social history of the individual child.

The purpose of this is to identify those children whose symptoms or disease are better understood within a social or sociopsychological framework.

Furthermore, the results of the investigation might have implications for the preventive work with an understanding of those changes which seem to have taken place in important social conditions and the role which stressful life events might have as a precipitant of disease.

ACKNOWLEDGEMENT

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REFERENCES

- 1 Apley J & McKeith R. *The child and his symptoms*. Blackwell Scientific Publications, Oxford 1968.
- 2 Christensen V. *Bølgeløst og bølgeslægt*. Munksgaard, Copenhagen 1956.
- 3 Christensen V. Trivselsproblemer og sygdomsreaktioner i førskolealderen. *Ugeskr Læger* 136: 1897 1974.
- 4 Coddington R D. The significance of life events as etiologic factors in the diseases of children. *J Psychosom Res* 16: 7 1972.
- 5 Coddington R D. The significance of life events as etiologic factors in the diseases of children II. *J Psychosom Res* 16: 20 1972.
- 6 Folke og boligværdien. Danmarks Statistik, Copenhagen 1973.
- 7 Gersten J C, Langner T S, Eisenberg J G & Simckla Fagan O. An evaluation of the etiologic role of stressful life change events in psychological disorders. *J Health Soc Behav* 18: 228 1977.
- 8 Helsinki II Declaration. *Ugeskr Læger* 138: 399 1976.
- 9 Robertson J. *Young children in hospital*. Tavistock Publications, Baltimore 1958.
- 10 Robing H N. *Deviant children grow up*. The Williams & Wilkins Company, Baltimore 1966.
- 11 Rosberg G. Social and psychological factors influencing hospital admission of children. *Acta Paediatr Scand Suppl* 196: 1969.
- 12 Rutter M. Psychosocial disorders in childhood and their outcome in adult life. *J R Coll Physicians London* 4: 211 1970.
- 13 Stocking M, Rothney W, Grosser G & Goodwin R. Psychopathology in the paediatric hospital. Implication for community health. *Am J Public Health* 62: 991 1972.

- 14 Straus P, Cottard N, Marzo Weyl B & Lenoir M. *L'hospitalisation des enfants. Une étude de pédiatrie sociale dans l'agglomération parisienne*. Monographie de l'Institut National d'Hygiène. No 73. Paris 1961
- 15 Aagaard J. Psykosocial belastning og sygdom hos børn. Metodediskussion. *Ugeskr Læger* 139 1961 1977
- 16 Aagaard J & Bro P. Psykosocial belastning og sygdom hos børn. Evaluering af pilot materiale. *Ugeskr Læger* 139 1966 1977
- 17 Aagaard J. *Admission to hospital. An analysis of the*

social history of children admitted to a paediatric department. Institute of Social Medicine. University of Aarhus. No 18. 1978

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SCREENING FOR HYPERLIPOPROTEINEMIA IN 10000 DANISH NEWBORNS

Follow up Studies in 522 Children with Elevated Cord Serum VLDL-LDL-Cholesterol

GUNNAR E. ANDERSEN, PER LOUS and BENT FRIIS HANSEN

From the Neonatal Department, Rigshospitalet and the Department of Clinical Chemistry, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

ABSTRACT Andersen G, E. Lous P and Friis-Hansen B (Neonatal Department, Rigshospitalet and Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark). Screening for hyperlipoproteinemia in 10000 Danish newborns. Follow up studies in 522 children with elevated cord serum VLDL-LDL-cholesterol. *Acta Paediatr Scand* 68:541, 1979. — Among 10440 newborns, 522 with upper 5 percentile values for very low density lipoprotein cholesterol in cord serum were selected for follow up studies. Follow up was possible in 446 of these 522 families (85%) and familial hypercholesterolemia (FH) was diagnosed in 11. In 273 of the 522 children, serum lipids were determined between the ages of 1 and 2 years and were now found to be normal except in the 11 children with FH. Furthermore, the serum lipids were compared in subgroups of these 273 children divided according to obstetric complications (i.e. low birth weight, perinatal asphyxia and antepartum betamethasone treatment) which may cause a rise in serum lipids at birth. No differences were found between these subgroups at the age of 1–2 years.

KEY WORDS Cholesterol, triglyceride, lipoproteins, children, screening, familial hypercholesterolemia.

Recently (1) we have shown that newborns with elevated cord serum VLDL-LDL-C can be identified by a turbidimetric estimation of cord serum VLDL-LDL and that besides FH also prematurity, perinatal asphyxia and antepartum betamethasone may cause a rise in cord serum VLDL-LDL-C.

In this paper we present data to elucidate 1) how many children with upper 5 percentile values for cord serum VLDL-LDL-C have FH, 2) if the serum lipids will later on become normal or remain elevated in children with elevated serum VLDL-LDL-C at birth and 3) if the serum lipids in later childhood are the same or different in subgroups of children with elevated serum VLDL-LDL-C at birth and who differed with respect to gestational age, perinatal asphyxia and betamethasone treatment.

MATERIALS

Between September 1975 and February 1977 cord blood was obtained as described earlier (2) from 10440 infants consecutively born in the 11 major obstetric departments in Copenhagen (St. Joseph's, Frederiksberg, Øresund, Glostrup and Gentofte Hospital and the Rigshospitalet). Cord serum VLDL-LDL was measured by the turbidimetric method (3) and 1879 of the 10440 (18%) with the highest VLDL-LDL values were selected for a more extended lipid and lipoprotein determination. Of these 1879 newborns, 522 (5%) with the highest VLDL-LDL-C levels were subsequently selected for follow up studies to ascertain whether they belonged to families with FH.

Abbreviations: FH = familial hypercholesterolemia, AGA = appropriate for gestational age, SGA = small for gestational age, TC = total cholesterol, TG = triglyceride, VLDL = very low density lipoproteins, LDL = low density lipoproteins, HDL = high density lipoproteins, VLDL-LDL = VLDL + LDL determined by turbidimetry, VLDL-LDL-C = VLDL-cholesterol + LDL-cholesterol determined enzymatically after CaCl₂ heparin precipitation, HDL-C = HDL-cholesterol calculated as the difference (TC) - (VLDL-LDL-C).

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Abbreviations FH = familial hypercholesterolemia AGA = appropriate for gestational age SGA = small for gestational age TC = total cholesterol TG = triglyceride VLDL = very low density lipoproteins LDL = low density lipoproteins HDL = high density lipoproteins VLDL-LDL = VLDL + LDL determined by turbidimetry VLDL-LDL-C = VLDL-cholesterol + LDL-cholesterol determined enzymatically after CaCl₂ heparin precipitation HDL-C = HDL-cholesterol calculated as the difference (TC) - (VLDL-LDL-C)

Table 1 Lipid and lipoprotein-cholesterol values (mmol/l) in 11 pairs of parent-child with familial hypercholesterolemia (FH)

Kindred	Age (years)	T-C (mmol/l)	VLDL+LDL-C (mmol/l)	LDL-C (mmol/l)	HDL-C (mmol/l)	TG (mmol/l)
I II 5	31	8.54	5.41	5.75	3.13	0.84
I III 5	At birth	7.87	1.58		1.4	0.61
I III 5	1 1/2	6.43	5.17	4.61	1.76	1.81
II I	22	8.89	6.35	5.67	2.54	1.79
II II 4	At birth	11	1.35		0.76	0.73
II II 4	1 1/2	7.16	5.27	4.72	1.94	1.55
III 6	23	11.06	9.45	8.96	1.61	0.60
III 4	At birth	4.6	1.69		2.57	0.90
III 4	1	7.57	4.78	4.55	2.74	0.93
IV II 1	9	11.16	9.43	8.88	1.73	1.37
IV III 1	At birth	7.96	1.61		1.35	0.59
IV III 1	1 1/2	8.45	6.78	6.47	1.67	0.90
V II 2	21	9.69	6.88	6.61	2.81	0.97
V III 3	At birth	3.79	1.77		2.07	0.47
V III 3	1	7.76	4.24	4.00	1.07	0.73
VI II 7	29	7.80	5.41	4.45	2.39	2.55
VI III 6	At birth	3.15	1.45		1.70	0.53
VI III 6	1 1/2	6.53	4.46	4.07	2.07	1.36
VII 9	8	8.0	5.56	4.73	2.64	1.69
VII II 14	At birth	1.90	1.45		0.45	0.64
VII II 14	1	7.11	5.31	5.01	1.80	1.36
VIII 2	38	9.17	6.03	5.97	3.09	0.63
VIII 4	At birth	3.89	2.45		1.44	0.64
VIII 4	1	7.8	5.6	5.76	1.66	1.57
IX 11	19	9.67	7.17	6.85	2.55	0.59
IX III 1	At birth	7.8	1.30		0.98	0.57
IX III 1	1 1/2	7.35	4.97	4.71	2.43	0.79
X III 5	31	9.51	7.41	7.17	2.10	0.74
X IV 6	At birth	2.47	1.33		1.09	0.30
X IV 6	1 1/2	7.21	5.16	5.00	0.5	0.78
XI II 1	4	7.41	4.79	4.18	3.13	0.59
XI III 1	At birth	7.4	1.54		1.70	0.83
XI III 1	2 1/2	6.93	5.05	4.73	1.88	1.03

On type II diet

FH is here defined as an autosomal dominantly inherited disease with a three generation vertical transmission of hypercholesterolemia (>95th sex and age adjusted value for serum T-C and/or LDL-C in normal). Supey's definition of FH arises by the finding of hypercholesterolemia in one of the parents or in the child after age 1 (4) and so serum lipids were examined in either the parents and/or the child.

In 76 cases (15%) no follow up was possible in either the parents or the child. 2 of these 76 children had died before age 1 and it was decided not to study their parents. The remaining 54 families were not interested in participating.

In 97 of the 54 families venous blood samples were taken from both parents after a 1-hour fast and in the mothers at least 3 months after birth. In 98 families venous blood samples were taken from 98 children after age 1 (73 1- years old, 25 over 3 years) after an 8-hour

fast. This meant that in 149 families therefore both parents and the child were examined in 148 families only the parents and in 149 families only the child was examined. 1 serum T-C was found to be elevated in either the parents or the child (>95th sex and age adjusted percentile values for normal Danes (5)) an extended serum lipid and lipoprotein determination was made at least three times and all available family members were asked to join the study and their serum lipids and lipoproteins were determined.

METHODS

The methods are the ones already described (7) except that at follow-up in parents and children serum T-C was measured enzymatically on a Greiner Selective Analyzer II as described by Borner & Klose (6).

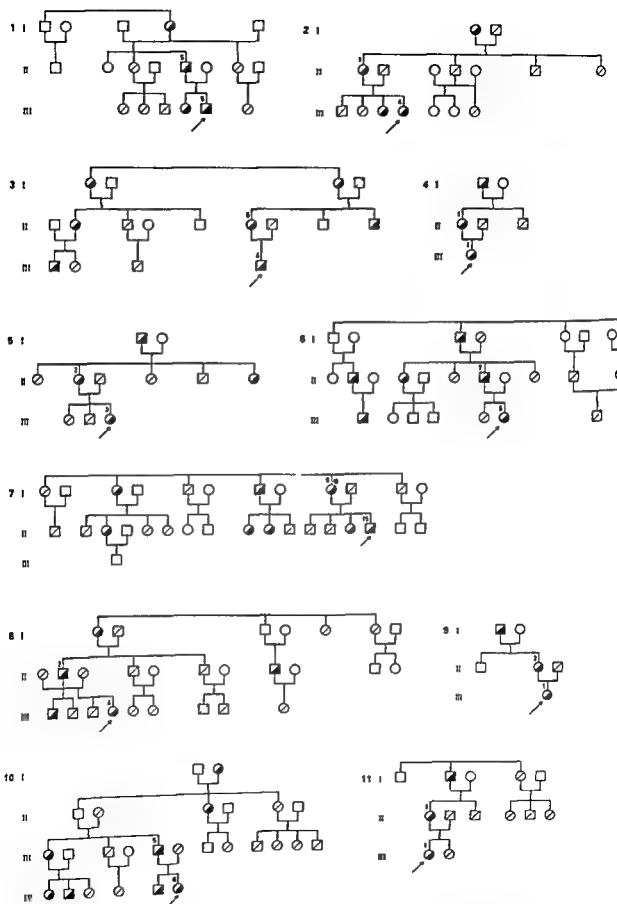


Fig. 1 The kindreds □ ○ Not sampled (deceased or abroad) ⊠ ⊡ died from premature myocardial infarction xanthomas
 ■ ● normal ◐ ◑ type IIa ↗ included in cord blood screening

cord serum VLDL-LDL-C and since a yet unknown number of children with FH may under certain circumstances be born with a normal cord serum VLDL-LDL-C level as shown earlier (1).

A minimum estimate however is possible since among 10440 consecutively born infants we have found 11 with a three generation vertical transmission of hypercholesterolemia which is generally accepted as an accurate criterion of FH (4). Thus a minimal incidence seems to be 0.11% which is close to 0.17% found by Tsang et al. (9) and 0.10% reported by Andersen & Friis Hansen (1) using the same diagnostic criteria. This makes FH the single most common inborn error of metabolism reported in Denmark so far.

Children with elevated cord serum VLDL-LDL-C irrespective of the genesis of this elevation (prematurity, perinatal asphyxia, betamethasone treatment) with the one important exception of FH had normal serum T-C and LDL-C at follow up between the ages of 1 and 2 years. The higher levels of serum TG and VLDL-C are probably explained by a fast of only 8 hours in these children as opposed to 10-12 hours in the reference group. This is an agreement with the findings of Boulton et al. (10) who reinvestigated 49 of 81 children with elevated cord serum LDL-C. At 4-12 months of age their serum T-C and LDL-C values were the same as in a control group. Hardell (11) reinvestigated an unreported number of children with elevated cord serum VLDL-LDL-C during their second year of life. In boys the same serum lipid levels were found as in a reference group whereas the girls had slightly higher T-C, VLDL-LDL-C and HDL-C values.

In conclusion the present study has shown that it was possible in at least 0.11% to make the diagnosis FH by a follow up study of the families of children who at birth had elevated serum VLDL-LDL-C. However the vast

majority of children with elevated VLDL-LDL-C values at birth have normal serum lipids at follow up.

ACKNOWLEDGEMENTS

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REFERENCES

- Andersen G E, Lous P & Friis Hansen B. Hyperlipoproteinemia in newborn infants. A study of 1075 families. *Acta Paediatr Scand* in press.
- Andersen G E & Friis Hansen B. Cord serum lipid and lipoprotein-cholesterol values in normal and betamethasone treated newborns of varying gestational age. *Acta Paediatr Scand* 66: 355, 1977.
- Andersen G E & Gry Nielsen H. Neonatal screening for hyperlipoproteinemia. Methods for direct estimation of cord serum VLDL+LDL. *Clin Chim Acta* 66: 79, 1976.
- Fredrickson D S, Goldstein J L & Brown M S. The familial hyperlipoproteinemias. In J B Stanbury, J B Wyngaarden & D S Fredrickson (eds) *The metabolic basis of inherited disease*. McGraw Hill, New York, 1978, p. 604.
- Andersen G E & Friis Hansen B. Neonatal diagnosis of familial type II hyperlipoproteinemia. *Pediatrics* 57: 714, 1976.
- Börner K & Klose S. Enzymatische Bestimmung des Gesamtcholesterins mit dem Greiner Selective Analyzer (GSA II). *J Clin Chem Clin Biochem* 15: 11, 1977.
- Siegel S. In S Siegel (ed) *Nonparametric statistics for the behavioral sciences*. McGraw Hill, New York, 1956, pp. 116 and 201.
- Andersen G E, Lifschütz C & Friis Hansen B. Dietary habits and serum lipids during first 4 years of life. A study of 95 Danish children. *Acta Paediatr Scand* in press.
- Tsang R C, Glueck C J, Fallat R W & Melies M. Neonatal familial hypercholesterolemia. *Am J Dis Child* 129: 83, 1975.
- Per oral communication.
- Hardell L. *Serum lipids and lipoproteins at birth and in early childhood*. Thesis, Uppsala, 1978.

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Table 2 Percentile values for serum lipids and lipoproteins in 265 children at follow up between ages 1-2 years

Maturity at birth	Serum lipid values (mmol/l)				
	5	10	50	90	95
I AGA ≥ 37 -42 weeks ($n=190$)					
T-C	3.48	3.69	4.75	5.77	6.08
VLDL+LDL-C	2.04	2.25	3.04	3.85	4.71
HDL-C	1.08	1.20	1.64	2.15	2.36
TG	0.53	0.59	0.84	1.42	1.70
II AGA < 37 weeks ($n=47$)					
T-C	3.53	3.69	4.63	5.64	6.14
VLDL+LDL-C	2.15	2.31	3.01	3.82	4.40
HDL-C	1.05	1.14	1.63	2.17	2.38
TG	0.49	0.58	0.87	1.01	1.10
III SGA ≥ 33 -42 weeks ($n=28$)					
T-C	3.40	3.50	4.65	5.68	5.85
VLDL+LDL-C	2.09	2.30	3.16	3.85	3.88
HDL-C	0.89	1.06	1.58	1.91	2.33
TG	0.45	0.52	0.83	1.34	1.80

Two cholesterol standards (A 77 and Pithonorm) were included in over 250 separate runs and the coefficients of variation were 3.1 and 4.0 resp.

The non-parametric statistical methods used are the same as the ones described by Siegel (7).

RESULTS

Among the 446 families available for follow up studies FH was found in 11. The 11 kindreds are depicted in Fig. 1 and the corresponding lipid and lipoprotein values in parent-child are given in Table 1. None of the 11 children had suffered perinatal asphyxia nor been given betamethasone. They were all delivered at term and were AGA. In all 11 parents common causes of secondary hyperlipemia were ruled out by repeated findings of normal glucose tolerance test, normal liver, thyroid and kidney function tests and normal immunoglobulins. Only one parent (II II 1), a 24 year old woman was known to have FH and had been treated with a type II diet, but no drugs for 1 1/2 years at the time we investigated the serum lipids of her daughter.

In Table 2 the percentile values are given for the serum lipids in 265 of the 273 children reinvestigated between the ages of 1 and 2 years. The values of 8 postmature newborns are left out.

Within each of the three groups (I mature, II premature and III small for date newborns) the lipid data of the four subgroups 1) no asphyxia, no betamethasone, 2) asphyxia, no betamethasone, 3) no asphyxia, betamethasone, 4) asphyxia, betamethasone have been pooled since no differences ($p > 0.05$) were found between I and 2 or between II and 4.

A comparison of the lipid values in groups I, II and III did not show any differences ($p > 0.05$). Neither were there any differences between the lipid values in boys and girls ($p > 0.05$). Furthermore, the lipid values in groups I, II and III were compared with the lipid values of a reference group of 64 normal Danish children aged 3-4 years (8). Serum T-C and LDL-C levels were similar in the two groups ($p > 0.05$). Serum TG and VLDL-C levels, however, were higher ($p < 0.001$) in the children aged 1-2 years.

DISCUSSION

The present study does not allow an accurate calculation of the incidence of FH in Danish children since follow up studies were only possible in 85% of the newborns with elevated

SMOKING DURING PREGNANCY—EFFECTS ON THE FETUS AND ON THIOCYANATE LEVELS IN MOTHER AND BABY

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ABSTRACT Meberg A Sande H Foss O P and Stenwig J T (Departments of Paediatrics Obstetrics and Gynecology Clinical Chemistry and Pathology Ullevål Hospital Oslo Norway) Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby *Acta Paediatr Scand* 68 547 1979.—Twenty-eight mothers smoking 10–20 cigarettes daily during pregnancy had significantly higher serum thiocyanate concentrations at delivery compared to 25 non smoking controls. The thiocyanate levels were positively correlated to cigarette consumption and inversely correlated to the birth weights of the infants. A highly significant correlation existed between serum thiocyanate levels of the mother and umbilical cord serum thiocyanate levels reflecting a nearly complete equilibration. The thiocyanate concentrations in human milk on the 4th day after delivery were considerably lower than the serum concentrations and no correlation existed between serum and milk concentrations. The infants of smoking mothers had significantly decreased weight and length at birth compared to infants of non smokers. Birth weights were 3344 ± 434 g and 3670 ± 304 g respectively ($p < 0.05$) and lengths 49.8 ± 1.7 cm and 51.1 ± 1.6 cm respectively ($p < 0.05$). No differences were found between smokers and non smokers in placental and umbilical cord histology and umbilical cord artery medial area. It is concluded that serum thiocyanate concentration in smokers may be used as an objective measure for smoke exposure and that maternal cigarette smoking acts as an exogenous factor which interferes with intrauterine development of the fetus in a dose related way.

KEY WORDS Cigarette smoke pregnancy infants thiocyanate

Maternal cigarette smoking during pregnancy interferes with fetal development. Smokers produce smaller babies (7 12 13 14 16) have a higher incidence of abortion (18) premature delivery (7 18) and perinatal mortality (7 13 18) than non smokers. Long term effects of maternal smoking on physical growth (8) and intellectual development (9) are found. Several factors are suggested to cause these effects on the fetus such as carbonmonoxide (6 17) and cyanide toxicity (1 16). Placental ultrastructural changes (2) as well as impaired placental blood flow (19) have also been described in smokers.

In smoking individuals cyanide is absorbed from the smoke and detoxicated to thiocyanate in the body (5 20). Increasing serum

thiocyanate levels have been found with increasing cigarette consumption (1 20).

The aim of the present investigation was to evaluate thiocyanate levels in mothers and their infants as an objective measure for smoke exposure and to investigate effects of maternal smoking on fetal size and on histology of the placenta and umbilical cord.

MATERIALS AND METHODS

Fifty three mothers (78 smokers and 25 non-smokers) and their newborn infants were included in the series. Consecutive smoking mothers were selected if they fulfilled the following criteria: healthy women aged between 20 and 30 years in their first or second uncomplicated pregnancy, a normal delivery at 39–42 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10 15 or 20 cigarettes daily for the whole pregnancy. The non smoking mothers selected

SMOKING DURING PREGNANCY—EFFECTS ON THE FETUS AND ON THIOCYANATE LEVELS IN MOTHER AND BABY

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ABSTRACT Meberg A, Sande H, Foss O P and Stenwig J T (Departments of Paediatrics, Obstetrics and Gynecology, Clinical Chemistry and Pathology, Ullevål Hospital, Oslo, Norway). Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby. *Acta Paediatr Scand* 68: 547-552, 1979.—Twenty-eight mothers smoking 10–20 cigarettes daily during pregnancy had significantly higher serum thiocyanate concentrations at delivery compared to 25 non-smoking controls. The thiocyanate levels were positively correlated to cigarette consumption and inversely correlated to the birth weights of the infants. A highly significant correlation existed between serum thiocyanate levels of the mother and umbilical cord serum thiocyanate levels, reflecting a nearly complete equilibration. The thiocyanate concentrations in human milk on the 4th day after delivery were considerably lower than the serum concentrations, and no correlation existed between serum and milk concentrations. The infants of smoking mothers had significantly decreased weight and length at birth compared to infants of non-smokers. Birth weights were 3344 ± 434 g and 3620 ± 404 g respectively ($p < 0.05$) and lengths 49.8 ± 1.7 cm and 51.1 ± 1.6 cm respectively ($p < 0.05$). No differences were found between smokers and non-smokers in placental and umbilical cord histology and umbilical cord artery medial area. It is concluded that serum thiocyanate concentration in smokers may be used as an objective measure for smoke exposure and that maternal cigarette smoking acts as an exogenous factor which interferes with intrauterine development of the fetus in a dose related way.

KEY WORDS Cigarette smoke, pregnancy, infants, thiocyanate

Maternal cigarette smoking during pregnancy interferes with fetal development. Smokers produce smaller babies (7, 12, 13, 14, 16), have a higher incidence of abortion (18), premature delivery (7, 18) and perinatal mortality (7, 13, 18) than non-smokers. Long term effects of maternal smoking on physical growth (8) and intellectual development (9) are found. Several factors are suggested to cause these effects on the fetus such as carbonmonoxide (6, 17) and cyanide toxicity (1, 16). Placental ultrastructural changes (2) as well as impaired placental blood flow (19) have also been described in smokers.

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The aim of the present investigation was to evaluate thiocyanate levels in mothers and their infants as an objective measure for smoke exposure and to investigate effects of maternal smoking on fetal size and on histology of the placenta and umbilical cord.

MATERIALS AND METHODS

Fifty three mothers (28 smokers and 25 non-smokers) and their newborn infants were included in the series. Consecutive smoking mothers were selected if they fulfilled the following criteria: healthy women aged between 0 and 30 years in their first or second uncomplicated pregnancy, a normal delivery at 39–47 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10, 15 or 20 cigarettes daily for the whole pregnancy. The non-smoking mothers selected

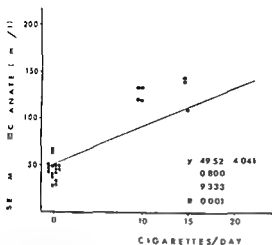


Fig 1 Relation between serum thiocyanate concentrations at delivery in 51 women and their smoking level during pregnancy

Blocks were taken from the cord membranes central and peripheral placental areas and processed in a routine fashion. In addition 2-3 cm long pieces of cord were taken 10 cm from the placental insertion and fixed in buffered 4% formalin (pH 7.4). Transverse sections were made and stained by the Verhoeff-van Gieson method to visualize the elastic lamina and the medial smooth muscle. The arteries were photographed through a microscope. In those cut at a right angle and with complete obliteration of the lumen the area between the internal lamina elastica and the outer margins of the media was calculated by an electronic morphometric calculator (Kontron Messgeräte GMBH) as a measure for the arterial medial muscle mass.

Details inquiry

A written inquiry was sent to the mother after discharge from the hospital concerning her weight 10 days after delivery, iron and vitamin supplementation and food intake during pregnancy.

Statistical methods

P-values were obtained by means of the Chi square test, Fisher's test and by simple linear regression analysis.

RESULTS

Table 1 presents the clinical and laboratory data concerning the mothers, the infants, the placentas and the umbilical cords in the two groups. Smoking mothers were of lower age than non smokers and the group contained more single women than the controls who were nearly all married. The groups were

equal with regard to parity of the mothers, mothers' weight and height before pregnancy, weight gain during pregnancy and weight gain of the mother herself as calculated from her weight 10 days after birth. The sex distribution of the infants was equal in both groups as was gestational age.

Significantly higher serum thiocyanate levels were present in smokers compared to non smokers. The thiocyanate concentration in serum fell slightly from just before delivery until the 4th day after delivery, however not significantly. In the 21 non pregnant non smoking controls, serum thiocyanate concentration was $55 \pm 15.5 \mu\text{mol/l}$ and similar to that in the pregnant non smokers ($p > 0.05$). The thiocyanate concentration in human milk was considerably lower than in serum and no difference existed between smokers and non smokers. No correlation was present between the serum and milk concentrations of thiocyanate ($p > 0.05$). Significantly increasing thiocyanate concentrations were found with increasing cigarette consumption (Fig 1).

A highly significant correlation existed between the thiocyanate concentration of the mother within 24 hours before delivery and the umbilical cord serum thiocyanate con-

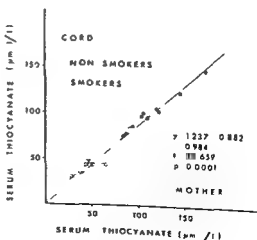


Fig 2 Relation between maternal serum thiocyanate concentrations and umbilical cord serum thiocyanate concentrations in 76 women smoking 10-20 cigarettes daily during pregnancy and in 25 non-smoking controls

Table 1 *Clinical and laboratory data on 28 smoking and 25 non smoking mothers the infants placenta and umbilical cords*Cigarette consumption ranged 10-70 cigarettes daily during pregnancy in the smoking group n =number of observation

	Smokers			Non smokers			<i>p</i>
	<i>n</i>	Range	Mean \pm S D	<i>n</i>	Range	Mean \pm S D	
Maternal age (years)	28	20-30	23.8 \pm 2.9	25	21-30	27 \pm 1.8	<0.05
Married	15	-	-	22	-	-	<0.05
Living together with the child's father	8	-	-	3	-	-	>0.05
Single	5	-	-	0	-	-	<0.05
Para 0/para 1	13/15	-	-	11/14	-	-	>0.05
Maternal height (cm)	28	158-181	166.9 \pm 5.6	25	156-178	167.5 \pm 5.5	>0.05
Maternal weight before pregnancy (kg)	25	49-83	59 \pm 9.5	23	45-85	59.3 \pm 8.7	>0.05
Total weight gain during pregnancy (kg)	24	6-21	13.2 \pm 3.9	23	8.3-24	15 \pm 4.2	>0.05
Weight gain of the mother alone (kg)	22	-2.5-16	4.4 \pm 4.5	20	-2-11	3.9 \pm 3.4	>0.05
Maternal serum thiocyanate at delivery (μ mol/l)	26	52-173	109.6 \pm 29.9	25	23-82	48.2 \pm 16	<0.05
Cord serum thiocyanate (μ mol/l)	26	50-149	99.2 \pm 27.2	25	24-82	43.3 \pm 13.5	<0.05
Maternal serum thiocyanate 4th day after delivery (μ mol/l)	24	49-154	100.3 \pm 27.8	25	24-81	43.6 \pm 13.2	<0.05
Hum in milk thiocyanate 4th day after delivery (μ mol/l)	24	4-53	15.6 \pm 13.2	24	3-62	18.1 \pm 15.6	>0.05
Gestational age (weeks)	28	39-42	40.4 \pm 1.1	25	39-42	40.6 \pm 1.0	>0.05
Infants sex ratio (male/female)	16/12	-	-	13/12	-	-	>0.05
Infant birth weight (g)	28	2480-4160	3344 \pm 434	25	2960-5150	3670 \pm 504	<0.05
Infant birth length (cm)	28	47-53.5	49.8 \pm 1.7	25	47-55	51 \pm 1.6	<0.05
Infant head circumference at birth (cm)	27	32.5-37	34.8 \pm 1.2	24	32.5-39	35.4 \pm 1.4	>0.05
Infant weight loss (g)	28	100-290	173.6 \pm 50.9	25	50-110	170.4 \pm 68.4	>0.05
Time for lowest weight (days after birth)	28	3-7	3.5 \pm 1.0	25	3-5	3.3 \pm 0.5	>0.05
Placental weight (with membranes) (g)	28	400-700	560 \pm 86.4	25	450-750	590 \pm 89.9	>0.05
Placental weight (without membranes) (g)	25	370-560	490 \pm 61	25	400-690	517 \pm 76	>0.05
Placental diameter (cm)	25	16.6-21	19.8 \pm 1.5	22	15.5-22.5	19.3 \pm 1.8	>0.05
Umbilical cord artery medullary area (mm ²)	21	1.61-3.17	2.32 \pm 0.51	17	1.47-3.44	2.42 \pm 0.59	>0.05

were the first non smoking women admitted for delivery after a mother in the smoking group and that fulfilled all the other selection criteria. An additional 21 healthy non pregnant non smoking women 21-30 years old (23.4 \pm 2.5 years mean \pm S D) were investigated for serum thiocyanate concentrations.

The mothers age marital status height weight before pregnancy and weight gain during pregnancy were registered from the hospital records as were weight length and head circumference of the infants at birth. Infant body weight was registered daily and the loss of weight after birth calculated.

Thiocyanate investigations

Venous blood samples were taken from the mothers within 24 hours before delivery and again on the 4th

day after delivery. Cord blood samples were taken at delivery from the placental part of the cord. A milk sample was collected from the mothers on the 4th day after delivery. Blood samples were rapidly centrifuged and all serum samples were stored at -20°C together with the milk samples. The serum and milk samples were analyzed for thiocyanate by an automated modification of the method of Pettigrew & Fell (15).

Histological investigations

Placentas were weighed after separating the cord at the placental insertion and also weighed after stripping off the membranes fixed in 4% formalin. The diameter of each placenta was registered as the mean of two angularly measured diameters. The placentas were studied grossly and cut in transverse sections 1.5 cm wide.

fetus of smoking mothers therefore may be exposed to toxic cyanide levels in utero

The lower concentrations of thiocyanate in human milk and the lack of correlation between serum and milk concentrations may be due to the handling of the thiocyanate ion in the mammary gland. Thiocyanate shows a physical and chemical similarity to halogen ions and behaves much like chloride ions in the body (20) which also are found in low concentration in human milk compared to serum concentration.

There has been discussion on whether cigarette smoking acts as an exogenous factor which interferes with intra uterine development of the fetus (1, 12, 13) or whether smoking women are a self selected group more likely both to smoke and to have infants with lower birth weights (10). In our study the maternal serum thiocyanate levels were inversely correlated to infant birth weight indicating that smoking itself interferes with fetal growth in a dose related manner. Similar inversely has been found in far greater series between birth weight and maternal smoking levels (12, 13). The growth retardation did not seem to be caused by differences in the two groups of nutritional factors during pregnancy (maternal weight gain), genetical potential for growth (maternal size) or marital status of the mothers.

Ultrastructural changes are found in umbilical cord vessels after maternal smoking during pregnancy (3, 4) as well as in placental vessels (2). In our study we did not investigate small changes and probably therefore were not able to show differences between smokers and non smokers.

Hypertrophy of medial smooth muscle is found in conditions associated with increased vascular resistance (11). If the decreased blood flow through the placenta found in smokers (19) is caused by an increased vascular resistance it might have been reflected in hypertrophy of smooth muscle in the umbilical cord arteries. We were however not able to demonstrate this with the method used.

ACKNOWLEDGEMENTS

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REFERENCES

- Andrews J. Thiocyanate and smoking in pregnancy. *Br J Obstet Gynaecol* 80: 810, 1973.
- Asmussen I. Ultrastructure of the human placenta at term. Observations on placentas from newborn children of smoking and non smoking mothers. *Acta Obstet Gynecol Scand* 56: 119, 1977.
- Asmussen I. Ultrastructure of human umbilical vessels. Observations on veins from newborn children of smoking and nonsmoking mothers. *Acta Obstet Gynecol Scand* 57: 253, 1978.
- Asmussen I & Kjellden K. Intimal ultrastructure of human umbilical arteries. Observations on arteries from newborn children of smoking and nonsmoking mothers. *Circ Res* 36: 579, 1975.
- Chung J & Wood J L. Oxidation of thiocyanate to cyanide catalyzed by hemoglobin. *J Biol Chem* 246: 555, 1971.
- Cole P V, Hawkins L H & Roberts D. Smoking during pregnancy and its effects on the fetus. *Br J Obstet Gynaecol* 79: 787, 1972.
- Comstock G W, Shah F K, Meyer M B & Abbey H. Low birth weight and neonatal mortality related to maternal smoking and socioeconomic status. *Am J Obstet Gynecol* 111: 53, 1971.
- Dunn H G, McBurney A K, Ingram S & Hunter C M. Maternal cigarette smoking during pregnancy and the child's subsequent development. I. Physical growth to the age of 6½ years. *Can J Public Health* 67: 499, 1976.
- Dunn H G, McBurney A K, Ingram S & Hunter C M. Maternal cigarette smoking during pregnancy and the child's subsequent development. II. Neurological and intellectual maturation to the age of 6½ years. *Can J Public Health* 68: 43, 1977.
- Hickey R J, Clelland R C & Bowers E J. Maternal smoking, birth weight, infant death and the self selection problem. *Am J Obstet Gynecol* 131: 805, 1978.
- Levin D L, Fidler I E, Morris F C & Tyson J. Morphologic analysis of the pulmonary vascular bed in infants exposed in utero to prostaglandin synthetase inhibitors. *J Pediatr* 97: 478, 1978.
- MacMahon B, Alpert N & Salber E J. Infant weight and parental smoking habits. *Am J Epidemiol* 87: 747, 1966.
- Meyer M B, Jonas M J & Tonascia J A. Perinatal events associated with maternal smoking during pregnancy. *Am J Epidemiol* 103: 464, 1976.
- Murphy J F, Mulchay R & Drumm J E. Smoking and the fetus. *Lancet* II: 36, 1977.
- Pettigrew A R & Fell G S. Simplified colorimetric determination of thiocyanate in biological fluids and its application to investigation of the toxic amblyopias. *Clin Chem* 18: 996, 1972.

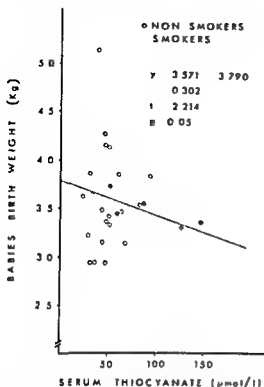


Fig. 3 Relation between maternal serum thiocyanate concentrations at delivery in 26 women smoking 10-20 cigarettes daily during pregnancy and in 25 non smoking controls and the birth weights of their infants

centration (Fig. 2) and also between maternal serum thiocyanate concentration on the 4th day after delivery and the cord serum level ($r=0.979$ $p<0.0001$).

A significant inversivity was found between birth weight and mother thiocyanate levels (Fig. 3). There was no statistically significant correlation between the mothers' estimated cigarette consumption and birth weight ($r=-0.217$ $p>0.05$).

The infants of smoking mothers had significantly lower weight and length at birth. These differences were also present when calculated for only the married women in the two groups. Weight loss after birth was equal in both groups of infants, as was the timing for the lowest weight after birth.

No difference was found on placental size (weight/diameter) between smokers and non smokers. Histological investigation of the placentas (25 placentas from each group) showed syncytial knots, small infarctions, small calcifications and fibrinoid necrosis of villi in most

placentas in both groups. These changes were not of any size or number suspect of any influence on placental function. In sections from the umbilical cords and membranes no pathological findings were present. The umbilical artery medial area was not different in smokers and non smokers.

The inquiry about food intake during pregnancy showed a varied and probably adequate food intake in all mothers. Twenty-five smokers and 23 non smokers used iron supplements and 25 smokers and 21 non smokers a multivitamin preparation during pregnancy.

DISCUSSION

Serum thiocyanate concentrations of smoking mothers were found to be positively correlated to their smoking levels (Fig. 1). This is in accordance with other investigations (1, 20). Pregnancy did not seem to influence the thiocyanate concentration as judged from our results in non smokers. The thiocyanate concentration of serum in smokers therefore may be used as an objective measure for the smoke exposure. However, non smokers also have a considerable and variable amount of thiocyanate in serum, mostly caused by the thiocyanate content in food (20). This limits the accuracy of the thiocyanate concentrations reflecting the smoking level.

The thiocyanate concentrations in cord blood serum were found to be nearly identical with maternal serum thiocyanate concentrations at delivery, as well as on the 4th day after delivery. Thus, cord serum thiocyanate seems to reflect the mother's smoking habits in the same way as her own serum thiocyanate concentration. Whether the fetal thiocyanate level reflects only passive diffusion of thiocyanate from the mother or also cyanide detoxication in the fetus itself remains unknown. However, a dynamic equilibrium exists between thiocyanate formed by detoxication of cyanide and reverse oxidation of thiocyanate to cyanide by enzymes present in red blood cells and other cells (5). The

SPONTANEOUS LABOUR AND ELECTIVE INDUCTION— A PROSPECTIVE RANDOMIZED STUDY

Behavioural Assessment and Neurological Examination in the Newborn Period

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KEY WORDS Newborn, behavioural assessment, neurological examination, elective induction, asphyxia.

The indications and methods for induction of labour have varied considerably, leading to difficulties in evaluating reports of pros and cons with this procedure. Induction of delivery on medical (clinical) indications, i.e. placental insufficiency or prolonged pregnancy, has been an accepted procedure for a long time. Opinions differ concerning induction at full term without clinical indications.

In previous retrospective studies the induction of delivery has been claimed to reduce the perinatal mortality and morbidity because of an intensified foetal monitoring and reduced frequency of postterm deliveries (12, 19, 21). Other authors have found an increased frequency of Dip I after induction and consider

it to be a risk for developing cerebral injuries (27). Martell et al. (20) have observed reduced pH in cord blood after induction with amniotomy. However, Alderman (1) found no difference in the frequency of low Apgar scores between spontaneous and induced labour using either low dose or titrated oxytocin techniques. Follow up studies of children born after elective induction have not demonstrated any negative effects compared with control groups (18, 22). There are only a few reports in which the effect of induced delivery has been studied prospectively. No studies have been published in which the neurological and behavioural state of the newborn infants have been examined after induced delivery. In the

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- 18 Rush D & Koss E H. Maternal smoking: A re-assessment of the association with perinatal mortality. *Am J Epidemiol* 96: 183, 1972.
- 19 Spira A, Fournier R, Grob J C, Dreyfus J & Schwartz D. Smoking during pregnancy and placental blood flow. *Proc 6th Congress of Perinatal Medicine*, Vienna 1978.
- 20 Storr K F. *Studies on thiocyanate in serum*. 1957. Medical Yearbook, University of Bergen.

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Table 2 Behavioural items in the Brazelton Neonatal Assessment Scale

Lability of skin colour and smiles are excluded

<i>Dimension 1</i> Interactive processes	Orientation inanimate visual (red ball) Orientation inanimate auditory (rattle) Orientation animate visual (face) Orientation animate auditory (voice) Orientation animate visual and auditory (face and voice) Alertness Cuddliness Consolability
<i>Dimension 2</i> Motor processes	General tone Pull to sit Motor maturity Defensive movements Hand to mouth activity Activity level
<i>Dimension 3</i> Organizational processes control of state	Response decrement to light Response decrement to rattle Response decrement to bell Response decrement to pinprick Peak of excitement Rapidity of buildup Lability of states Irritability Self-quieting activity
<i>Dimension 4</i> Organizational processes response to stress	Tremulousness Startles

chlone 40 mg (pethidine—Atarax®) by intramuscular injection and pudendal block with 70 ml 0.5% Mepivacaine chloride (Carbocain®).

Procedure and delivery

Immediately after delivery the newborn infant was placed on the delivery bed at the level of the vaginal outlet. Exactly 60 sec after delivery the cord was clamped and a blood sample from the umbilical vein was drawn for pH analysis. 0.7 mg methyl ergometrine (Methergin®) was then given to the mother.

Examination of the infant

The infants were evaluated according to Apgar at 1 and 5 min. During the second day a maturity assessment using external characteristics was performed (14). The infants were examined on days 1 and 5 with the Brazelton Neonatal Behavioural Assessment scale (9). These examinations were done without knowledge of which group the infants belong to and in a calm surrounding. Roughly 1 hour after feeding. Standard administration procedures were followed but two items, lability of skin colour and smiles, were not analyzed. All infants were examined by the same examiner (J.L.) who had been trained to reliability by the Brazelton group. The Brazelton Examination

Table 3 Grouping of items in the neurological examination

Figures in parentheses are possible scores

Items measuring muscular tonus (8–31)

Spontaneous posture supine position (1–3)
Resistance against passive movements: body and neck (1–4)
Resistance against passive movements: arms (1–4)
Resistance against passive movements: legs (1–4)
Recoil arms (1–4)
Traction response: arms (1–4)
Traction response: head (1–4)
Head control: vertical position (1–4)

Items measuring excitability (9–33)

Optical blink reflex (1–3)
Acoustic blink reflex (1–3)
Patellar reflex (1–4)
Palmar grasp (1–4)
Withdrawal reflex (1–4)
Rooting reflex (1–4)
Ability to suck (1–4)
Moro tremor (1–3)
Spontaneous motility (1–4)

Optimal items (Items where optimal response is possible to state)

The items measuring muscular tonus (see above)
The items measuring excitability (see above)
Moro tremor excluded
Face expression (1–7)
Cry (1–4)
Symmetry in body position (1–2)
Eyes following a red ball (1–3)
Dolls eye test (1–7)
Pupils reaction to light (1–3)
Glabella reflex (1–7)
Moro reflex (1–4)
Crawling (1–4)
Placing response (1–2)
Automatic walking (1–3)

Total neurological score (36–126)

Optimal items (see above) (7–97)

Neurological symptoms

Drowsiness (1–3)
Jitteriness (1–7)
Convulsions (1–7)
Others (1–7)

Fontanel tension (1–6)

Position and movements of eyes (1–7)

Side difference = resistance against passive movements (1–7)

Asymmetric Moro (1–2)

Moro tremor (1–3)

assesses the infant's behaviour with regard to 27 items in a nine point scale (see Table 2). The items were organized in four dimensions according to Als et al (7, 3): I. Interactive processes, i.e. the infant's capacity for orientation to external stimuli and II. respond to social stimuli. 2. Motor processes, i.e. the infant's ability to control motor behaviour and muscle tonus. 3. Organization processes

Table 1 The number and the age of the women in induced and spontaneous labour

Mean and \pm SD

	Induced labour		Spontaneous labour	
	n	Mean age	n	Mean age
Primiparae	18	22.4 \pm 4.5	18	23.6 \pm 3.4
Multiparae	23	26.3 \pm 4.0	21	25.0 \pm 3.1

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1. Maternal age between 18 and 30 years for primiparae and 18 to 35 for multiparae.
2. The last menstrual period normal and known. Regular menstrual periods before the actual pregnancy. Women using hormonal contraceptives should have had at least three normal periods after completed medication.
3. Previous pregnancies and deliveries normal with birth weights between 3000 and 4000 grams.
4. Normal symphysis-fundus distance and weight gain according to gravidogram (32).
5. The present pregnancy normal and the foetus in vertex presentation.
6. Normal pelvic outlet according to clinical examination.

Patients fulfilling these criteria were examined within one week before expected date of delivery. The condition of the cervix was estimated in a ten point scale modified after Bishop (5-31). If a pelvic score of at least 5 points for primiparae and at least 4 points for multiparae was found, the patient was invited to participate in the study. The date of planned delivery was decided to ± 2 days from the date of expected delivery. If the woman agreed to participate she was randomly put into one of two groups. In group I deliveries were induced and in group II deliveries were allowed to start spontaneously. Patients in whom the labour started spontaneously earlier than the date of planned delivery were excluded from the investigation. If the pregnancy in

group II was prolonged more than 14 days from the date of estimated delivery, the delivery was induced according to the routine clinical indications in the departments. These patients belonged to the original group.

112 women fulfilling the criteria given above agreed to participate: mean age 24.5 ± 4.0 years. 13 in the induction group (group I) and 12 in the spontaneous group (group II) were delivered before the date of planned delivery. 3 mothers in group II had prolonged pregnancies. As the number of these patients were few and did not affect the results they were included in the study. There were 7 Caesarean sections due to disproportion between the foetal head and the maternal pelvis. These two infants were in good condition but were excluded from the investigation. The first five infants in the study were not completely examined and were excluded. A total of 80 infants were examined: 41 in group I and 39 in group II. The distribution and the age of the women in the groups are shown in Table 1. Seventeen of the infants were born and examined in Linköping, the others in Motala.

Induction of delivery—group I

On the morning of the day of planned delivery, amniotomy was performed through an amnioscope and a catheter for registration of intraamniotic pressure was inserted. A scalp electrode was applied on the foetal head for registration of foetal heart frequency. The registrations were performed using the Hewlett Packard or Corometrics equipment.

Oxytocin was infused intravenously using the Cardif infusion pump system Mark II (11-15). An alarm for intrauterine pressure higher than 80 mmHg and for uterine spasm (intraamniotic pressure > 35 mmHg for more than 120 sec) was attached to the system. The infusion was started 15 min after amniotomy with an initial infusion rate of 1 mU/min and was increased continuously until the intensity of the contractions reached at least 35 mmHg and a frequency of at least one contraction every 15 sec. The infusion was stopped immediately after birth.

Spontaneous delivery—group II

The women were asked to come to the delivery ward as soon as labour started. External CTG registration was performed until an established labour could be demonstrated. This was thought to be the situation when the frequency of contractions was at least one every 5 min, the cervix was effaced at least 50% and dilated at least 2 cm for primiparae and at least 3 cm for multiparae. Amniotomy was now done: a catheter for intrauterine registration of pressure and a scalp electrode was applied. The registrations were then performed in the same manner as in group I.

The progress of labour was followed every hour with regard to the degree of dilatation of the cervix and the position of the head in the pelvis and was registered in a partogram.

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Crawling (1–4)
Placing response (1–2)
Automatic walking (1–3)

Total neurological score (36–16)

Optimal items (see above) (7–9)

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Drowsiness (1–3)
Jitteriness (1–7)
Convulsions (1–7)
Others (1–7)
Fontanel tension (1–6)
Position and movements of eyes (1–2)
Side difference in resistance against passive movements (1–7)
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Table 5 The four dimensions in the Brazelton Scale (Brazelton Neonatal Assessment Scale 1973) in neonates born after induced and spontaneous labour

The table shows the results on day 1 and 5 of the number and percentage of neonates in each performance score (mean and standard deviation (1 S D)) of performance score. Performance score 1 means very good performance, average performance and 3 poor performance.

Performance score	Day 1				Day 5			
	Induced labour		Spontaneous labour		Induced labour		Spontaneous labour	
	n	%	n	%	n	%	n	%
Dimension 1	n=41		n=36		n=41		n=38	
1	6	14.6	4	11.1	10	24.4	7	18.4
2	31	75.6	24	66.7	29	70.7	29	76.3
3	4	9.8	8	22.2	2	4.9	2	5.3
Mean	1.95±0.50		2.11±0.58		1.80±0.51		1.87±0.49	
Dimension 2	n=41		n=35		n=41		n=38	
1	0	0	2	5.7	2	4.9	4	10.5
2	30	73	21	60.0	39	95.1	34	89.5
3	11	26.8	1	2.8	0	0	0	0
Mean	2.7±0.45		2.9±0.57		1.95±0.2		1.89±0.31	
Dimension 3	n=8		n=25		n=6		n=22	
1	5	62.5	7	28.0	3	50.0	3	13.6
2	2	25.0	18	72.0	18	69.2	16	72.7
3	1	12.5	0	0	1	16.7	3	13.6
Mean	1.86±0.45		1.7±0.46		2.08±0.46		2.00±0.44	
Dimension 4	n=41		n=36		n=41		n=38	
1	30	73.2	9	25.0	40	97.6	36	94.7
2	11	26.8	7	19.4	1	2.4	2	5.3
Mean	1.54±0.90		1.39±0.80		1.05±0.31		1.11±0.45	

dimension 2 $t=6.11$ $p<0.001$ dimension 3 $t=2.23$ $p<0.05$ dimension 4 $t=4.14$ $p<0.001$. The differences between day 1 and 5 indicate that on day 5 more infants showed a better orientation to external stimuli and a

higher alertness (dimension 1) more infants had a better ability to control motor behaviour (dimension 2) more infants had less tremulousness and startles (dimension 4) but fewer infants had a good state control (dimension 3).

Table 6 Mean and standard deviation for the sum of four different groupings in the neurological examination

A high value in tonus score means a low muscular tonus. A high value in excitability score means low degree of excitability.

	Day 1		Day 5	
	Induced labour (n=41)	Spontaneous labour (n=39)	Induced labour (n=41)	Spontaneous labour (n=39)
Tonus score (optimal=14) range 8-30				
Excitability score (optimal=14) range 9-33	14.05±0.70	13.86±0.59	14.1±0.55	14.2±0.90
Total number of optimal items (max 7)	14.83±1.9	14.83±1.54	14.0±0.54	13.97±0.70
Total score range 37-116	55.41±1.59	54.89±1.85	56.37±1.0	61.14±1.50
	57.93±3.0	57.7±8.1	51.56±1.40	51.61±1.61

Table 4 Birth weight, lowest weight during the first week, gestational age, gestational age according to maturity assessment, Apgar scores and pH in umbilical vein in induced and spontaneous labour

Mean \pm S.D.

	Induced labour (n=41)	Spontaneous labour (n=39)
Birth weight, grams	3634 \pm 453	3700 \pm 499
Lowest weight during the first week, grams	3376 \pm 436	3444 \pm 458
Gestational age, days	290 \pm 1	285 \pm 3
Gestational age according to maturity assessment, days	290 \pm 12	283 \pm 8
Apgar score 1 min	8.8 \pm 0.7	9.0 \pm 0.4
Apgar score 5 min	9.9 \pm 0.4	9.9 \pm 0.3
pH umbilical vein	7.35 \pm 0.07	7.33 \pm 0.07

is the infant's ability to control and organize his state (4). Physiological stability in response to stress. For each infant a performance score was calculated ranging from 1 to 3 points for each dimension (3), whereby 1 means a very good performance, 2 a fair performance and 3 a poor performance.

A neurological examination based mainly on Brecht & Beintema (23) was carried out on day 1 and 5. At the same occasion is the behavioural assessment. The examination included 37 items, most of them evaluated in a four degree scale. Some of these items were grouped in those evaluating mainly muscle tonus or excitability (Table 3). A tonus score and an excitability score were calculated according to Schulte et al. (16). The number of optimal responses and a total neurological score were also calculated for each infant.

All infants were breast fed freely and a naked weight was recorded daily.

Statistical methods

Conventional statistical methods were employed. Student's *t* test was used throughout.

The investigation was approved by the Ethical Committee of the University of Linköping.

RESULTS

The results for the two clinics have been pooled, as there were no differences between them. The statistical analyses were made with regard to parity, but as there were no differences in these respects primiparae and multiparae have been combined in the further presentation of the results.

One infant to a primipara was born in the occiput posterior position, all the others in the occiput anterior position. There were two vacuum extractions in the spontaneous group, one because of prenatal isphyxia and one because of uterine inertia. There was a third vacuum extraction in the induction group due to prenatal isphyxia. One infant in the induction group was evaluated as preterm at the external maturity assessment. There were no differences between the groups in the use of analgesia in primiparae. However 87% of multiparae in group I and 43% of multiparae in group II were given pethidine chloride-hydorizine chloride during labour ($p < 0.01$).

The mean values for gestational age and maturity assessment did not differ between the two groups. There were no statistically significant differences in birth weight, weight reduction, Apgar scores at 1 and 5 min or in cord pH (see Table 4).

In the behavioural assessment mean values for each item were compared for the two groups. The two groups were also compared with regard to a performance score within the four dimensions. There was a considerable drop out within dimension three which includes items measuring the infant's ability to control and organize his state (17). 53 infants could be evaluated on day 1 and 48 on day 5. The reason for drop out was that items measuring the response decrement to external stimuli could not be evaluated because the infant changed his state of arousal during this period of the examination. The drop out for the other items was small.

There were no statistically significant differences between the two groups regarding the individual items. The results of the four dimensions are described in Table 5. The distribution and percentage of infants in performance score 1-3 for the four dimensions did not show any significant differences between the groups. Comparison of the distribution of infants of each performance score on days 1 and 5 gave significant differences for all the four dimensions (dimension 1: $t = 2.72$, $p < 0.01$).

Table 5 The four dimensions in the Brazelton Scale (Brazelton Neonatal Assessment Scale 1973) in neonates born after induced and spontaneous labour

The table shows the results on day 1 and 5 of the number and percentage of neonates in each performance score, mean and standard deviation (S.D.) of performance score. Performance score 1 means very good performance, 2 average performance and 3 poor performance.

Performance score	Day 1				Day 5			
	Induced labour		Spontaneous labour		Induced labour		Spontaneous labour	
	n	%	n	%	n	%	n	%
Dimension 1	n=41		n=36		n=41		n=39	
1	6	14.6	4	11.1	10	24.4	7	18.4
2	31	75.6	31	66.7	29	70.7	29	76.3
3	4	9.8	8	22.2	2	4.9	2	5.3
Mean	1.95±0.40		2.11±0.58		1.80±0.41		1.87±0.48	
Dimension 2	n=41		n=35		n=41		n=38	
1	11	26.8	7	20.0	7	17.1	4	10.5
2	30	73.2	28	80.0	34	82.9	34	89.5
3	11	6.8	1	3.3	0	0	0	0
Mean	2.0±0.45		2.0±0.57		1.9±0.40		1.89±0.31	
Dimension 3	n=8		n=6		n=6		n=6	
1	5	62.5	7	83.3	3	50.0	3	50.0
2	1	12.5	1	16.7	1	16.7	1	16.7
3	2	25.0	0	0	2	33.3	2	33.3
Mean	1.86±0.45		1.77±0.46		2.08±0.56		2.00±0.54	
Dimension 4	n=41		n=36		n=41		n=38	
1	30	73.2	9	25.0	40	97.6	36	94.7
2	11	26.8	27	75.0	1	2.4	2	5.3
Mean	1.44±0.90		1.39±0.80		1.05±0.31		1.11±0.45	

dimension 2 $t=6.11$ $p<0.001$ dimension 3 higher alertness (dimension 1) more infants
 $t=2.23$ $p<0.05$ dimension 4 $t=4.14$ $p<$ had a better ability to control motor behaviour
 0.001) The differences between day 1 and 5 (dimension 2) more infants had less tremu-
indicate that on day 5 more infants showed a lousness and startles (dimension 4) but fewer
better orientation to external stimuli and a infants had a good state control (dimension 3)

Table 6 Mean and standard deviation for the sum of four different groupings in the neurological examination

A high value in tonus score means a low muscular tonus. A high value in excitability score means low degree of excitability.

	Day 1		Day 5	
	Induced labour (n=41)	Spontaneous labour (n=39)	Induced labour (n=41)	Spontaneous labour (n=39)
Tonus score (optimal=14) range 8-30	14.05±0.70	13.86±0.59	14.17±0.55	14.2±0.90
Excitability score (optimal=14) range 9-33	14.83±1.9	14.81±1.54	14.0±0.54	13.97±0.70
Total number of optimal item (max=4)	5.41±1.59	4.89±1.85	6.3±1.0	6.14±1.50
Total score range 3-116	5.93±3.30	5.37±2.81	51.46±1.40	51.61±1.61

There were no differences between the two groups in this respect.

The neurologic investigation gave no significant differences between the two groups for the tonus score, the excitability score, the total number of optimal responses or the total score (see Table 6). Comparing the results on day 1 and 5, there was no difference in tonus score ($t=1.86$ n.s.). There was, however, a difference in the excitability score ($t=4.81$, $p<0.001$) showing that the infants on day 1 were less excitable. On the first day the infants had fewer optimal points ($t=6.09$, $p<0.001$) and a higher total score ($t=5.98$, $p<0.001$). There were no differences between the two groups, induced or spontaneously delivered, in this respect.

DISCUSSION

In this investigation, other facts than the induction procedure which could possibly influence the infant's neurological status, have as far as possible been kept constant. Factors such as the gestational age (25) and analgesia (13) influence the neurological function postnatally. Through the selection procedure of the mothers, the gestational ages were well known and all infants were probably born at term. This is important since an unexpected preterm delivery after induction might lead to an increased risk for the infant (6, 7, 16). Although the obstetric analgesia was restricted, there was a difference in multiparity in the use of pethidine chloride-hydroxyzine chloride. This fact did not effect the results as there were no differences between the groups.

The time of amniotomy has also been standardized in the spontaneous group. Because of the early amniotomy, the spontaneous group is comparable with the induced group in this respect. The amniotic pressure was recorded in all deliveries. This procedure probably reduces the risk for nitrogenic hypertonic labour and thus the risk for foetal asphyxia. The foetal heart rate was recorded continuously during delivery. There were no differ-

ences in foetal heart rate changes. Apgar score and cord pH between the two groups. These results have been discussed in detail elsewhere (31).

In order to demonstrate a possible negative influence on the neurological function of the newborn infant, we used a well standardized neurological examination technique based mainly upon Prechtl & Beintema (23). This method has been used in several neonatal studies (24) in which early neurological findings could be correlated to risk pregnancies and risk deliveries. The results of the present study did not show any significant differences between the groups. We examined the infants on two occasions, day 1, in order to better evaluate the effects of the delivery per se and day 5, when the possible influences of the delivery should be minimal (4, 23). We actually found differences between day 1 and 5 showing that the infants on day 1, as a rule, were less excitable. These results are in agreement with Beintema (4). The infants on day 1 also had a lower optimal score and a higher total neurological score, indicating the normal developmental course during the first days of life described by Beintema (4). The two groups did not differ, however, with regard to changes from day 1 to 5.

According to some authors (3, 28), a neurological examination technique is not sensitive enough to show minor dysfunction of the central nervous system of the newborn. A behavioural assessment is claimed to detect such minor dysfunctions better. The Brazelton Scale has been used both clinically and as a research instrument in investigations of obstetric anaesthesia and its effects on newborn behaviour (8, 30), on narcotic addicted newborns (28, 29) and on nutritionally deprived newborn infants (10). This assessment, which is rather time consuming, is said to evaluate the infant's ability to react to different stimuli and to evaluate the infant's ability to control his state. The reproducibility of the Brazelton's method is not very well documented and factors such as the author's experience and the

circumstances surrounding the examination might influence the results. In the present examination we tried to reduce this variation and only one trained person performed the behavioural assessment and the neurological examination of all infants. The behavioural assessment was standardized with respect to the surroundings and the time of day when the examinations were performed. Our own reproducibility was equivalent to Brazelton's (9). Further views on the method and its reproducibility will be published separately. As mentioned earlier, there was a considerable drop out in dimension 3. However, this drop out did not differ between the two groups.

There were no significant differences between the two groups within any of the four dimensions. However, the examinations showed that the number of infants with good performance increased from day 1 to 5 within dimensions 1, 2 and 4. In dimension 3, where most items reflect the infant's temperament and excitability (arousal) (17), the results showed a more normal distribution of infants in each performance score on day 5 compared to day 1. This agrees with the results of the neurological investigation in which the infants in general were less excitable on day 1 as mentioned above.

The present investigation thus showed that induction of labour at full term after a normal pregnancy, a normal foetal growth and a ripe cervix had no negative effects on the infant's behavioural and neurological state. Other prerequisites for this result were known gestational age, induction with oxytocin guided by intrauterine pressure registration and a continuous foetal heart rate recording throughout the delivery.

REFERENCES

- Alderman B. Dangers of oxytocin-induced labour to fetuses. *Br Med J* IV 44: 19-4.
- Al H. Tronick E, Adamson L & Brazelton T B. The behavior of the full term but underweight newborn infant. *Dev Med Child Neurol* III 590: 1976.
- Al H. Tronick E, Lester B & Brazelton T B. The Brazelton Neonatal Behavioral Assessment Scale (NBAS). *J Abnorm Psych* 5: 715: 1977.
- Beintema D J. A neurological study of newborn infants. *Clinics in Developmental Medicine* No 78. Heinemann London 1968.
- Bishop E. Pelvic scoring for elective induction. *Obstet Gynecol* 24: 66: 1964.
- Blacow M, Smith M N, Graham M & Wilson R G. Induction of labour. *Lancet* I 217: 1975.
- Booth J H & Kuryak V B. Elective induction of labour: a controlled study. *Can Med Assoc J* 103: 45: 1970.
- Brackbill Y, Kane J, Manniello R L & Abramson H. Obstetric premedication and infant outcome. *Am J Obstet Gynecol* 118: 377: 1974.
- Brazelton T B. Neonatal Behavioral Assessment Scale. *Clinics in Developmental Medicine* No 40. Heinemann London 1973.
- Brazelton T B, Tronick E, Lechus A, Lasky R E & Klein R E. The behavior of nutritionally deprived Guatemalan infants. *Dev Med Child Neurol* 19: 364: 1977.
- Butterworth M J. Automated incremental oxytocin infusion in the induction of labor. *Obstet Gynecol* 44: 238: 1974.
- Cole R A, Howie P W & Macnaughton M C. Elective induction of labour. A randomised prospective trial. *Lancet* I 767: 1975.
- Dubowitz V. Neurological fragility in the newborn. Influence of medication in labour. *Br J Anaesth* 47: 1005: 1975.
- Finnstrom O. Studies on maturity in newborn infants II. External characteristics. *Acta Paediatr Scand* 61: 74: 1977.
- Francis J G, Turnbull A C & Thomas F F. Automatic oxytocin infusion equipment for induction of labour. *J Obstet Gynaecol Br Commonw* 77: 594: 1970.
- Keettel W C, Randall J H & Donnelly M M. The hazards of elective induction of labor. *Am J Obstet Gynecol* 75: 496: 1958.
- Lester M H, Emory E K & Hoffman S L. A multivariate study of the effects of high risk factors on performance on the Brazelton Neonatal Assessment Scale. *Child Dev* 47: 515: 1976.
- McBride W C, Lyle J G, Black B, Brown C & Thomas D. A study of five year old children born after elective induction of labour. *Med J Aust* 2: 456: 1977.
- McNay M H, McIlwaine G M, Howie P W & Macnaughton M C. Perinatal deaths: analysis by clinical cause to assess value of induction of labour. *Br Med J* I 34: 1977.
- Martell M, Belizan J M, Nieto F & Schwarcz R. Blood acid-base balance at birth in neonates from labors with early and late rupture of membranes. *J Pediatr* 89: 363: 1976.
- Martius G. Terminierung der Entbindung und perinatale Sterblichkeit. *Dtsch Med Wochenschr* 101: 489: 1976.
- Niswander K R, Turoff B H & Romans J.

Developmental status of children delivered through elective induction of labor *Obstet Gynecol* 27 15 1966

23 Prechtl H F R & Beintema D The neurological examination of the fullterm newborn infant *Clinics in Developmental Medicine* No 12 Heinemann London 1964

24 Prechtl H F R Neurological sequelae of prenatal and perinatal complications *Br Med J* iv 763 1967

25 Saint Anne Diquissies S La maturation neurologique du prématuré *Etud Neo natal* 4 71 1955

26 Schulte F J Schrempf G & Hinze G Maternal toxemia, fetal malnutrition and motor behavior of the newborn *Pediatrics* 48 871 1971

27 Schwarz R I Belizin J M Cifuentes J R Curdso J C Marques M B & Caldeyro Barcia R Fetal and maternal monitoring in spontaneous labors and in elective inductions *Am J Obstet Gynecol* 170 356 1974

28 Soule III A H Stundley K & Copins S A Clinical uses of the Brazelton Neonatal Scale *Pediatrics* 54 583 1974

29 Strauss M E Iessen Firestone J K Starr R H & Ostrea F M Behavior of narcotics addled newborns *Child Dev* 46 887 1975

30 Tronick E Wise S Als H Adamson L Scanlon J & Brazelton T B Regional obstetric anesthesia and newborn behavior: Effect over the first ten days of life *Pediatrics* 58 94 1976

31 Tylleskär J Finnstrom O Hedenskog S Leyon I & Rydén G Spontaneous labour and elective induction—a prospective randomized study: Effects on mother and foetus *Acta Obstet Gynecol Scand* in press

32 Westin H Gravidiogram and foetal growth: Comparison with biochemical supervision *Acta Obstet Gynecol Scand* 56 273 1977

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EFFECT OF INTRAUTERINE NUTRITIONAL DEPRIVATION ON NEUROMOTOR BEHAVIOUR OF THE NEWBORN

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ABSTRACT Bhatia V P, Katiyar G P and Agarwal K N (Department of Paediatrics Institute of Medical Sciences Varanasi India) Effect of intrauterine nutritional deprivation on neuromotor behaviour of the newborn. *Acta Paediatr Scand* ■ 561 1979.—The neurological maturation in 25 newborn babies born to severely undernourished mothers was studied by evaluating muscle tone and excitability status. These mothers had weight below the 25th percentile expected for height, haemoglobin less than 80 g/l and serum albumin less than 30 g/l. Twenty three babies born to healthy mothers were studied as control. The babies of undernourished mothers demonstrated gross intrauterine growth retardation. There occurred parallel reduction in placental weight and its protein content. The neuromotor behaviour of these newborns showed significant alteration in the performance of most reflexes resembling normal motor behaviour of preterm infants. As many as 72% newborns could be classified as hypotonic and 56% hyporexitable. However, no newborn demonstrated hypertonia or hyperexcitability. The parameters of neuromotor assessment were found to have no correlation with the birth weight in both undernourished as well as control groups. These observations suggest that muscle tone and excitability are better indices of maturation of central nervous system than the birth weight. It seems that the neurological evaluation becomes unreliable in babies who suffer from intrauterine nutritional deprivation.

KEY WORDS Newborn, muscle tone, muscle excitability, maternal undernutrition.

Studies on normal preterm and low birth weight newborn babies have led to a general consensus that neurologic maturation proceeds in accordance with the gestational age and is unaffected by premature birth and adverse intrauterine influences (3, 10, 18, 19). This theory has received additional support from studies of electroencephalographic maturation, nerve conduction velocity and evoked potentials in the newborn (4, 5). However, follow up studies of intrauterine growth retarded babies have demonstrated serious motor and intellectual deficiencies suggesting that this concept should be applied cautiously to newborns who have suffered severe intrauterine insult, especially nutritional deprivation (2). In the present study muscle tone and excitability status of newborns who suf-

fered from severe intrauterine undernutrition have been assessed by quantitative evaluation of various reflexes.

MATERIAL AND METHODS

Subjects

Twenty five newborn babies of severely undernourished mothers who had haemoglobin and serum albumin levels less than 80 g/l (mean 50.5 S.E. \pm 7.0 g/l) and 25 g/l (mean 17.2 S.E. \pm 1.0 g/l) respectively and weighed less than the 5th percentile of the expected weight for height (mean 40.8 S.E. \pm 0.89 kg) were taken for the study. The mean height of these mothers was 149.3 \pm 1.06 cm. Maternal height and weight were measured 1-7 days after delivery. Twenty three newborn babies of healthy mothers having regular antenatal check up, haemoglobin more than 100 g/l, serum albumin more than 35 g/l and height and weight above 145 cm (155.1 \pm 0.97 cm) and 45.0 kg (55.9 \pm 1.35 kg) respectively served as controls. In both the groups mothers with uncomplicated antenatal and obstetrical history and known date of con-

Table 1 Placental weight protein content and birth weight in control and undernourished groups (mean \pm S L)

n = number of observations. Values in parentheses indicate the range

Parameters	Control group (<i>n</i> = 23)	Undernourished group (<i>n</i> = 25)
Placental weight (g)	451 \pm 13.0 (397.0-547.0)	299 \pm 11.9 * (256.0-400.0)
Placental protein content (mg/g wet tissue)	62.9 \pm 1.00 (52.0-68.0)	57.1 \pm 1.04 (48.8-68.8)
Birth weight (g)	3 140 \pm 71.7 (2 720-3 690)	2 034 \pm 50.5 (1 500-2 270)

* $p < 0.001$

ception only were included. Mothers with pathological conditions responsible for blood loss (e.g. antepartum haemorrhage), proteinuria (e.g. toxemia, pyelonephritis, nephrosis, etc.) or conditions likely to affect foetal growth (e.g. chronic infections, tuberculosis, heart disease, hypertension and diabetes) were excluded from the study. All the newborns studied were full term having gestation of 280 ± 2 days with an uneventful delivery and hospital stay. Placentae were also studied so as to evaluate the effect of intrauterine undernutrition on the foeto-maternal unit.

Methodology

A. Neurological assessment The neurological examination was performed 2-4 days after birth and 14 to 24 hours after feeding in a room having a comfortable temperature and humidity. None of the neonates had any feeding problems and all were on normal bottle or breast feeding.

The method of examination and the scoring was adopted from Prechtl & Buitens (16). Further some complex motor phenomena like spontaneous motor activity of face and general body rooting, sucking and cry were also scored on the four point scale as attempted by Schulte (20). All responses were obtained during optimal behaviour state only.

Various reflexes and responses in the newborn can be grouped into (a) those dependent on phasic neuronal activity and therefore reflecting excitability status of the newborn and (b) those dependent on constant neuronal discharge i.e. related to muscle tone and posture. Both excitability and muscle tone status are considered to be important aspects of newborn behaviour indicating maturity of the central nervous system and/or its dysfunction. Neurological evaluation was thus conducted from two aspects.

(a) *Responses reflecting the muscle tone status of the newborn* These comprised of posture of arms and legs, resistance to passive movement of neck, trunk, jaw

arms and legs, recoil of forearms, traction response, posture in prone suspension and supporting reaction.

(b) *Reflexes and responses reflecting excitability status of the newborn* These included spontaneous motor activity of general body and face, glabellar reflex, optical and acoustic blinks, biceps, triceps, knee, ankle and jaw jerks, palmar and plantar grasps, crossed extensor reflex, withdrawal, rooting, sucking, Gallant's response, Moro's response, stepping and cry.

The various reflexes and responses were scored as follows:

Response	Score
No response	0
Weak response/resistance	1
Good response/resistance	2
Strong/eager response/flow threshold/tremulousness	3

An account was also made for asymmetrical responses.

The sum of individual scores in each group served as quantitative indices of the excitability and muscle tone status of the newborn. Accordingly babies were classified as hypo/hyper-excitable or hypo/hyper-tone when their score varied more than ± 2 S.D. from the mean excitability and muscle tone scores respectively of 11 normal newborns.

B. Biochemical studies Maternal blood samples were collected before delivery and serum was separated immediately. Wet placental weight was recorded after trimming the membranes to the margin and cutting the cord at inversion. Tissue protein as well as serum albumin and total proteins were estimated by the method described by Lowry et al. (13). Since blood glucose, calcium and magnesium levels affect the neuromotor excitability as well as the EEG pattern, these parameters were analysed in the newborn serum immediately after the recordings were taken. The methods adopted for blood sugar (glucose oxidase method), serum magnesium (Tit in yellow method) and serum calcium (Tisdall method as modified by Clark & Collip) are described by Frank et al. (9).

RESULTS

All the newborns studied were apparently healthy on general and systemic examination and none had any congenital anomaly. None suffered from any birth injury or birth asoxia and their hospital stay (mean 6 ± 3 days) was uneventful. Babies born to undernourished mothers demonstrated significantly low means for birth weight, placental weight and placental protein content as compared to the well nourished group (Table 1). The correlation coefficient (*r*) between placental weight and newborn weight were calculated in the control and undernourished groups separately.

Table 2 *Percentage of babies showing different scores for individual reflexes grouped under Musck Tone Status*

Values in parentheses indicate the sample size

Reflexes	Control group (73) Score				Undernourished group (25) Score			
	0	1	2	3	0	1	2	3
1 Posture of arms	—	—	100	—	4	36	60	—
Posture of legs	—	—	95.6	4.4	—	36	64	—
<i>Response to passive movement</i>								
3 - Neck	4.4	65.2	30.4	—	20	76	4	—
4 - Trunk	—	30.4	69.6	—	4	84	12	—
5 - Jaw	—	76.1	73.9	—	4	72	24	—
6 - Arms	—	—	65.2	34.8	—	76	24	—
7 - Legs	—	—	34.8	65.2	—	16	84	—
8 Recoil of forearms	—	21.7	60.9	17.4	32	56	12	—
9 Recoil of legs	4.4	—	47.8	47.8	8	0	7	—
10 Traction response	—	17.4	78.3	4.4	40	40	0	—
11 Head control	13.0	6.1	5.7	8.7	48	40	12	—
12 Posture in prone suspension	—	13.0	87.6	4.4	20	60	16	4
13 Supporting reaction	—	13.0	46.6	30.4	8	72	12	8

The values were found to be $+0.44$ ($p < 0.01$) and $+0.32$ ($p < 0.01$) respectively. The mean head circumference of the babies belonging to undernourished group was 33.2 ± 0.38 cm which did not differ significantly from control group (34.1 ± 0.27).

Four newborns born to undernourished

mothers showed severe intrauterine growth retardation with birth weights of 1500, 1668, 1804 and 1814 g, all were kept in low birth weight nursery for a period of 3 weeks or more. Their stay in nursery was free from any significant illness with satisfactory weight gain.

Table 3 *Percentage of babies showing different scores for individual reflexes grouped under Excitability Status*

Values in parentheses indicate the sample size

Reflexes	Control group (73) Score				Undernourished group (25) Score			
	0	1	2	3	0	1	2	3
1 Spontaneous motor activity (Gen)	—	4.4	95.7	—	—	32	68	—
2 Spontaneous motor activity (Face)	—	—	100	—	—	4	76	—
3 Glabellar reflex	—	1.7	73.9	4.4	—	56	44	—
4 Optical blink	—	8.7	73.9	17.4	—	60	8	1
5 Acoustic blink	—	13.0	56.5	30.4	—	68	3	—
6 Biceps jerk	—	30.4	69.6	—	—	68	3	—
7 Triceps jerk	4.4	60.9	34.8	—	16	84	—	—
8 Knee jerk	—	4.4	95.7	—	—	72	24	4
9 Palmar grasp	—	8.7	73.9	17.4	—	72	64	12
10 Plantar grasp	4.4	4.4	65	26.1	—	44	44	1
11 Crossed extensor reflex	—	43.5	47.8	8.7	—	72	0	8
12 Withdrawal reflex	—	4.4	95.7	—	—	72	68	8
13 Rooting response	—	13.0	78.3	8.7	—	5	44	4
14 Sucking	—	4.4	86.9	8.7	—	72	7	4
15 Jaw jerk	—	47.8	57.7	—	—	64	36	—
16 Moro response	—	—	78.3	21.7	—	8	64	8
17 Ankle jerk	—	56.5	43.5	—	—	60	40	—
18 Stepping	—	34.8	56.5	8.7	8	64	24	4
19 Gallant's reflex	4.4	39.1	56.5	—	16	48	36	—
20 Cry	—	—	1.7	78.3	—	16	7	12

Table 4 *Muscle tone and excitability scores in babies born to control and undernourished mothers (mean \pm S.E.)*

Values in parentheses indicate the sample size

Group	Control (73)	Under nourished (25)
Muscle tone score	25.74 \pm 0.74	16.24 \pm 0.62
Excitability score	37.87 \pm 0.81	30.69 \pm 0.86
Modal muscle tone score	27	15
Modal excitability score	39	30

** $p < 0.001$

Neurological behaviour

(i) *Muscle tone status* The newborns of the undernourished group demonstrated poor response to most of the reflexes which reflect

Muscle tone status and 40% showed partial to complete limp posture as compared to the control newborns. The lower limbs were observed to be less affected in terms of passive resistance and recoil. The reduced tone of neck, trunk, arms and jaw resulted in an absence of head control in 48% and limp head was found during prone suspension in 80% as compared to corresponding figures of 13% for both these reflexes in the control group. The response to supporting reaction revealed that axial muscle tone was reduced in the undernourished group (Table 2).

The muscle tone score for the newborns of the undernourished group was significantly lower than the control group ($p < 0.001$, Table 4). The modal muscle tone scores for the control and undernourished groups were 27 and 15 respectively. On the basis of these scores, 18 (72%) newborns belonging to the undernourished group could be classified as hypotonic (score less than 18).

(ii) *Excitability status* One third to one fourth of the newborns in the undernourished group showed poor spontaneous motor activity in general and facial activity as compared to that observed in the control group. The babies born to undernourished mothers had poor performance for almost all the reflexes grouped for evaluating excitability.

However various tendon reflexes (biceps, triceps, knee and jaw jerks), Gallant's response and cry were less affected. In 28% of the newborns belonging to the undernourished group Moro's response was incomplete with an absent or weak adductor phase. Except for knee jerk, other tendon jerks were difficult to elicit in a fairly large number of control newborns as well (Table 3).

The calculated excitability scores were 37.9 \pm 0.81 and 30.7 \pm 0.86 respectively for newborns of the control and undernourished groups, the difference being significant ($p < 0.001$). The modal scores were found to be 39 and 30 for these groups respectively. On this basis, 14 (56%) newborns of undernourished mothers had excitability score less than 2 S.D. of the mean score of the control group (score 30) and therefore were considered hypoeccitable or apathetic. No newborn was found to be hyperexcitable (Table 4).

The muscle tone and excitability scores in different birth weight groups demonstrated identical means for muscle tone and excitability scores for the newborns weighing between 2000–2500 g and those below 2000 g (Table 5). However the means in both groups were lower than the means observed in newborns above 2500 g birth weight. The levels of blood sugar, serum calcium and magnesium were found to be identical in newborns of both groups.

DISCUSSION

In this study babies of severely undernourished mothers showed intrauterine growth

Table 5 *Distribution of muscle tone and excitability scores according to birth weight (mean \pm S.E.)*

Values in parentheses indicate mean weights

Birth weight (g)	n	Muscle tone score	Excitability score
> 500 (3 140)	23	25.74 \pm 0.74	37.87 \pm 0.81
2 000–2 500 (2 146)	13	16.69 \pm 0.92	31.47 \pm 1.47
< 2 000 (1 870)	12	15.75 \pm 0.88*	30.0 \pm 1.04

*** $p < 0.001$

retardation as evident by the findings of low birth weight and reduced placental size. This has been observed by other workers and ascribed to reduced protein biosynthesis (12-21).

Neurological status

Available studies have demonstrated that the neuromotor development of the newborn proceeds in accordance with the conceptional age and remains unaffected in preterm or small for dates babies. Graziani et al (11) selected a group of reflexes which mature during early organogenesis and are not likely to be affected by nutritional deprivation or factors operative in the later part of pregnancy. It is also likely that simple studies of reflex patterns eliciting their presence may not indicate the maturity status since responses may be qualitatively modified. Further studies have not classified their group of low birth weight infants and therefore not only the cause-effect relationship is lost but some otherwise normal low birth weight infants get included along with pathologically growth retarded neonates (6, 18, 19).

Prechtl (17) correlated the obstetrical risk factors with the neuromotor development and concluded that the mean neurological score decreased as the number of obstetrical complications increased. Farr & Mitchell (7) and Michaels et al (14) also demonstrated altered neuromotor maturation in low birth weight babies. The studies in babies of toxæmic mothers known to suffer pathological intrauterine growth retardation demonstrated significant hypotonia in these newborns (20). These workers were of the opinion that neuromotor maturation is likely to be affected if the materno-foetal supply line is jeopardized in any pathological condition.

In the present study babies of severely undernourished mothers on assessment for quality of the responses and the cumulative scores for various grouped reflexes demonstrated (i) significant hypotonia (72%) (ii) marked hypoexcitability (56%) and (iii) modi-

fication of responses in several reflexes viz limp posture, poor recoil of limbs, incomplete Moro's and crossed extensor responses etc. This is compared to the babies born to well nourished mothers. These features observed in undernourished newborns were close to the observations made by earlier workers in preterm babies (1, 6, 19). However, it is difficult to say whether associated anaemia along with generalised protein-energy deprivation also affects neuromotor maturation by chronic intrauterine hypoxia.

The muscle tone and excitability scores were similar amongst the babies weighing less than 2000 g and those weighing between 2000-2500 g (Table 5). When these scores were analysed in relation to the birth weight gestation being full term in all the newborns studied, no relation could be demonstrated between the two though both neuromotor development and birth weights were retarded. This suggests that evaluation of muscle tone and excitability are better indices when assessing the maturity of the central nervous system.

The critical analysis of neuromotor behaviour of the newborns who suffered from intrauterine undernutrition revealed that the reflexes mediated by polysynaptic pathways viz optical and acoustic blinks, crossed extensor reflex, rooting, sucking, Moro's and stepping responses were affected much more than those reflexes mediated through monosynaptic pathways. Various tendon reflexes, muscle tone also is dependent on a complex polysynaptic neural system involving cerebellum, reticular formation and various related nuclei. Animal studies have demonstrated defective cerebellar growth, deficient dendritic arborization and alterations in reticular formation (8, 15). These observations offer a possible explanation for the above results.

The present study concludes that intrauterine undernutrition results in altered activity of the brain as evident by muscle tone and excitability status. So far, no study is available on severe maternal undernutrition and other authors have taken small-for-dates babies

from pregnancy disorders like toxæmia anaemia due to interpartum haemorrhage smoking twinning etc (2 7 14 17 20). Still the consensus emerges that retardation of neuromotor maturation occurs during altered pregnancy states and maternal undernutrition. Therefore any assessment of neurological status to evaluate the gestational age in such babies is likely to be fallacious.

REFERENCES

- 1 André Thomas Chesni Y & St Anne Dargassies S. The neurological examination of the infant. *Clinics in Developmental Medicine* No 1 Spastics Society Heinemann London 1960
- 2 Bhakoo O N, Kaur S, Niring A, Verma S K, Chopra I S & Gupta A N. Neonatal neurological examination: perinatal factors, subsequent development and evaluation of a prognostic risk score. *Indian Pediatr* 14: 263 1977
- 3 Dotson E & Desmond M M. The evaluation of muscle tonus in the newborn. *Neurology (Minneapolis)* 14: 464 1964
- 4 Dreyfus Brivic C & Minkowski A. Electroencephalographic maturation and low birth weight. In P Kellaway and I Peterson (eds) *Neurological and EEG correlative studies in infancy*. Grune and Stratton New York 1964 p 49
- 5 Ellingson R J & Lindley D B. Brain waves and cortical development in newborns and young infants. *Am Psychol* 4: 248 1964
- 6 Farr V. Estimation of gestational age by neurological assessment in first week of life. *Arch Dis Child* 43: 357 1968
- 7 Farr V & Mitchell R G. Effect of birth weight on maturity scoring. *Develop Med Child Neurol* 9: 745 1967
- 8 Fish I & Winick M. The effect of malnutrition on the regional growth of developing rat brain. *Exper Neurol* 25: 534 1969
- 9 Frankel S, Reitman S & Sennowirth A C. *Gradwohl's Clinical laboratory methods and diagnosis*. C V Mosby Company St Louis 1970 7th edition
- 10 Gesell A A & Amatruda C S. In H Knecht and B Pasamanick (eds) *Developmental Diagnosis*. Harper and Row Publishers New York 1964 3rd edition
- 11 Griziani L J, Weitzman E D & Velasco M S. Neurologic maturation and auditory evoked responses in low birth weight infants. *Pediatrics* 41: 483 1968
- 12 Lager F M, Driscoll M G & Munro H A. Comparison of placentas from two socioeconomic groups. I Morphometry. *Pediatrics* 60: 24 1977
- 13 Lowry O H, Rosenbrough N J, Farr A L & Randall R J. Protein measurement with Folin phenol reagent. *J Biol Chem* 193: 265 1951
- 14 Michaelis R, Schulte F J & Nolte R. Motor behaviour of small for gestational age newborn infants. *J Pediatr* 76: 208 1970
- 15 Platt B S, Heard C M C & Steward R J C. Experimental protein calorie deficiency. In H R Munro and J H Allison (eds) *Mammalian protein metabolism*. Academic Press New York 1964 vol p 446
- 16 Prechtl H F R & Beintema D. Neurological examination of full term newborn infant. *Clinics in Developmental Medicine* No 12 Spastics Society Heinemann London 1964
- 17 Prechtl H F R. Neurological sequelae of prenatal and perinatal complications. *Br Med J* 1: 763 1967
- 18 Robinson R J. Assessment of gestational age by neurological examination. *Arch Dis Child* 41: 477 1966
- 19 Saint Anne Dargassies S. La maturation neurologique du prématuré. *Etud Neonatal* 4: 71 1954
- 20 Schulte F J. Maternal toxæmia, foetal malnutrition and motor behaviour of the newborn. *Pediatrics* 49: 871 1971
- 21 Sen S & Agarwal K N. Placental alterations in relation to maternal socio-economic status. *Indian Pediatr* 12: 865 1975
- 22 Winick M. Cellular growth of human placenta. III. Intrauterine growth failure. *J Pediatr* 71: 390 1967

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EVIDENCE OF RIBOFLAVIN DEPLETION IN BREAST FED NEWBORNS AND ITS FURTHER ACCELERATION DURING TREATMENT OF HYPERBILIRUBINEMIA BY PHOTOTHERAPY

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ABSTRACT Hovi L, Hekali R and Shmes M A (Children's Hospital University of Helsinki and Finnish Blood Transfusion Service Helsinki Finland) Evidence of riboflavin depletion in breast fed newborns and its further acceleration during treatment of hyperbilirubinemia by phototherapy. *Acta Paediatr Scand* 68 567 1979.—Phototherapy in the treatment of newborns with hyperbilirubinemia resulting in degradation of bilirubin also appears to have other photodynamic effects on metabolism. We studied flavin adenine dinucleotide (FAD) saturation of erythrocyte glutathione reductase which should reflect riboflavin nutritional status in 28 healthy newborns and followed 37 newborns with hyperbilirubinemia prior to the start of and during phototherapy. The results indicate that healthy newborns on human milk feeding relatively poor in riboflavin have evidence of a transient riboflavin depletion soon after birth. This effect is made more pronounced by phototherapy and partially prevented by parenteral or oral administration of moderate amounts of riboflavin.

KEY WORDS Breast feeding hyperbilirubinemia newborn phototherapy red blood cell enzymes riboflavin depletion

Phototherapy has an established role in the treatment of newborns with hyperbilirubinemia (21). This kind of exposure to high intensity light resulting in degradation of bilirubin also appears to have other less beneficial photodynamic effects on metabolism. Mild symptoms and signs are reported to be surprisingly common in jaundiced newborns receiving phototherapy including fever, diarrhea, irritability and skin rash (8). Fever and exposure of skin have been related to potential dehydration (16, 22), acid base balance disturbances (15) and subsequent circulatory responses (17). Diarrhea has been associated with deficiencies of intestinal enzymes such as disaccharidase (6) and lactase (1). Effects have also been reported on metabolism of nuclear DNA (20) and red blood cells (3) and even on the blood concentrations of various constituents including platelets (14), lipids (11) and several hepatic enzymes (5, 18). However, some of the above mentioned findings have only been observed in low birth

weight infants or in animal studies. Some long term sequelae related to growth and development have been suggested although these findings have been controversial (8). However, a bronze baby syndrome (12) and visual complications (7) are well documented although the latter are preventable by simple means. Recent data also indicate that infants with hemolytic disease such as glucose 6 phosphate dehydrogenase deficiency may not respond to phototherapy and increased hemolysis may result from the treatment (13).

Potentially correctable side effects of phototherapy are for example those which result in deficiencies of light sensitive vitamins such as riboflavin (10, 24). In this study we found some evidence of transient riboflavin depletion both in healthy and jaundiced newborns fed with breast milk during the first weeks of life and explored further the evidence of riboflavin depletion in jaundiced newborns during phototherapy and its prevention by administration of parenteral or oral riboflavin.

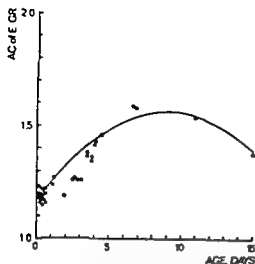


Fig. 1 Evidence of transient riboflavin depletion in healthy newborns and in newborns with hyperbilirubinemia prior to the start of phototherapy. Significance of the correlation between activity of erythrocyte glutathione reductase (AC of E GR) and age was high ($p < 0.001$).

SUBJECTS AND METHODS

The subjects were 37 consecutive full term newborns who were referred to the Helsinki Children's Hospital with hyperbilirubinemia (over $170 \mu\text{mol/l}$ ($10 \text{ mg}/100 \text{ ml}$)) but without evidence of a hemolytic disease or other major medical problem. In addition 28 healthy newborns were studied from 0 to 14 days as controls.

The newborns with hyperbilirubinemia were treated by phototherapy for periods from 1 to 7 days. The therapy was started at an average age of 3.5 days with a total range from 1 to 8 days. Two newborns were subsequently exchange transfused after which they were excluded from these data.

During phototherapy the newborns were undressed and only their eyes were shielded. There was a continuous exposure to light except during nursing. A bank of six natural white bulbs (Airm IXC) each of 20 W were kept at a distance of 35 cm above the baby. Each bulb was tested for its intensity at the intervals of three months and replaced if the intensity of visible light was less than 4000 lux. No additional shields were used since the lamps yielded only a trace amount of ultraviolet irradiation.

All subjects were fed with banked or fresh breast milk from birth through the period of the study.

The first 10 of the 37 newborns with hyperbilirubinemia received no riboflavin supplementation during phototherapy. Subsequently they randomly received riboflavin either intramuscularly ($n=8$) or orally ($n=10$) or no supplementation ($n=9$). Thus a total group of 19 newborns was not treated with riboflavin. The vitamin (riboflavin N_1 phosphate) was administered twice a day at a dose of 0.5 mg/kg/day . The administration was started immediately or within 8 hours after the initiation of phototherapy and continued through its duration.

Flavin adenine dinucleotide (FAD) saturation of erythrocyte glutathione reductase (E GR) is considered a

specific indicator of riboflavin nutritional status (4). A total of 54 blood samples was obtained from 37 newborns with hyperbilirubinemia prior to the start of phototherapy. In addition 94 samples were drawn at about 4 hour intervals during phototherapy to check the E GR. About half of the latter ($n=49$) were from newborns receiving riboflavin treatment. The group of healthy newborns was sampled only once. The blood was drawn in ACD solution kept at 4°C until centrifugation and washed twice with isotonic saline solution. The hemolysate was kept at -20°C until analyzed (7). The tests with and without FAD were performed concurrently. The results were expressed as activity coefficients (AC) where values above 1.0 indicate riboflavin deficiency (4).

The correlations were calculated by parabolic regressions. Statistical significances were also determined by Student's t test.

RESULTS

The AC values of E GR of the healthy newborns and of the newborns with hyperbilirubinemia prior to the start of phototherapy did not differ from one another and were thus used as control values ($n=82$) (Fig. 1). Accordingly hyperbilirubinemia per se had no influence on the activity of the enzyme. However the values were age dependent and increased during the first week of life. This rise reached statistical significance ($p < 0.001$). The mean values were slightly lower again during the second week of life as shown by the regression line in Fig. 1.

Phototherapy for hyperbilirubinemia further accentuated the increase in the AC values.

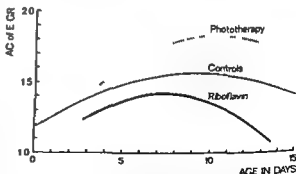


Fig. 2 Further acceleration of riboflavin depletion after initiation of phototherapy in newborns with hyperbilirubinemia and its prevention by riboflavin treatment. Significances of the differences are indicated in the text.

(Fig. 2) This difference reached statistical significance over the control values after 5 days of age ($p < 0.0025$). The effect was dependent on the duration of phototherapy ($p < 0.0025$) although it became pronounced within 24 hours. The mean values increased further (by the nonlinear regression line) from 1.52 at 24 hours to a maximum of 1.74 at 4 days of phototherapy respectively.

The treatment with riboflavin resulted in a marked decrease in the AC values ($p < 0.001$) to mean levels which were below the control values at any age (Fig. 2). This effect was similar in the newborns who were treated either intramuscularly or orally although the mean values of the former group were slightly (about 0.2 AC units) but insignificantly lower after 6 days of age. For these reasons the values of both treatment groups were pooled in Fig. 2.

DISCUSSION

The results show that the AC values of II GR increased in any group of newborns during the first week of life and that the newborns with hyperbilirubinemia receiving phototherapy showed a further increase. This effect was partially prevented by additional treatment with moderate amounts of riboflavin administered either parenterally or orally. Only these AC values reached a mean of 1.0 by two weeks of age, a value which should represent a normal mean. These observations suggest that phototherapy results in evidence of riboflavin depletion and confirm and further extend those reported by Gromisch and his associates (10).

In addition we found that even in healthy newborns there was some evidence of a transient depletion soon after birth. It is of interest that this finding might be explained by the exclusive feeding of human milk in our subjects since the concentration of riboflavin is relatively low in human milk, about 0.36 mg/l (20). In the two previous studies the authors did not report this phenomenon (10, 24). How-

ever their subjects were fed with infant milk formulas which contain about three times more riboflavin than human milk. These considerations indicate that newborns on human milk feeding might be more sensitive to the phototherapy induced riboflavin depletion. Further, the level of riboflavin in human milk may be marginal for the needs of every healthy newborn soon after birth.

Babies under phototherapy had no distinct symptoms or signs which could have been correlated with the degree of enzyme activity. However, flavin nucleotides serve as co-enzymes in several major metabolic pathways. Thus it remains possible that phototherapy might cause harmful metabolic changes. In accord with this possibility there is evidence for altered tryptophan metabolism during phototherapy, probably through riboflavin deficiency (19). It is not known however whether other metabolic pathways requiring FAD are affected, for instance the catabolism of purine nucleotides.

Further information is also needed to determine whether riboflavin supplementation might be beneficial in healthy human milk fed newborns and in those receiving phototherapy. A potential disadvantage of riboflavin treatment is suggested by *in vitro* studies which indicate that light exposed riboflavin may also have potential side effects (23).

In many hospitals phototherapy is liberally administered and only a few newborns with some hyperbilirubinemia may avoid it. However, the numerous reports including our findings tend to indicate various potential side effects. It remains to be determined whether the risks outweigh the benefits in most of the infants subjected to treatment.

REFERENCES

1. Bakken A F. Intestinal lactase deficiency as a factor in the diarrhea of light treated jaundiced infants. *N Engl J Med* 294: 615, 1976.
2. Beutler E, Blume K, G Kaplan J C, Lohr G M, Ramot M & Valentine W N. International Committee for Standardization in Haematology. Re-

- commended methods for red cell enzyme analysis *Br J Haematol* 35 331 1977
- 3 Blackburn M G Orzalesi M M & Pigram P Effect of light on fetal red blood cells *in vivo* *J Pediatr* 80 640 1972
 - 4 Cooperman J M Cole H E Gordon M & Lopez R Erythrocyte glutathione reductase as a measure of riboflavin nutritional status of pregnant women and newborns *Proc Soc Exp Biol Med* 143 326 1975
 - 5 Dicoiu Voutetakis C & Anagnostakis D Phototherapy and serum LH levels in newborns *Pediatr Res* 10 876 1976
 - 6 Dinari G Dium F Cohen M I & McNamara H Effect of phototherapy on intestinal disaccharidase activity in the rat *J Pediatr Res* 11 443 1977
 - 7 Dobson V Cowett R M & Riggs I A Long term effect of phototherapy on visual function *J Pediatr* 86 555 1975
 - 8 Drew J H Murrige K J Bayle V V Bajraszewski E & McNamara J M Phototherapy. Short and long term complications *Arch Dis Child* 51 454 1976
 - 9 Fomon S J & Filer L J Milk and formulas *In* S J Fomon (ed.) *Infant nutrition* W B Saunders New York 1974 2nd ed p 359
 - 10 Gromisch D S Lopez R Cole H S & Cooperman J M Light (phototherapy)-induced riboflavin deficiency in the neonate *J Pediatr* 90 118 1977
 - 11 Hadjigeorgiou E Triliouri D Trichopoulou A Kriskarellis D & Nicolopoulos H Influence of phototherapy on the serum lipids of jaundiced newborn infants *J Pediatr Res* 17 690 1978
 - 12 Kopelman A E Brown R S & Odell G H The bronze baby syndrome. A complication of phototherapy *J Pediatr* 91 466 1972
 - 13 Kopelman A E Ey J L & Lee H Phototherapy in newborn infants with glucose 6-phosphate dehydrogenase deficiency *J Pediatr* 93 497 1978
 - 14 Maurer H M Fratini M McWilliams N B Kirkpatrick H Draper D Haggins J C & Hunter C R Effect of phototherapy on platelet counts in low birth weight infants and on platelet production and life span in rabbits *Pediatrics* 57 506 1976
 - 15 Mueller D G Kirkpatrick B V Stoerner J R Hunter C R & Maurer H M Effect of phototherapy on acid base balance in low birth weight infants *Pediatr Res* 10 478 1976
 - 16 Oh W & Karecki H Phototherapy and insensible water loss in the newborn infant *Am J Dis Child* 174 230 1972
 - 17 Oh W Yano A C Hanson J S & Lind J Peripheral circulator response to phototherapy in newborn infants *Acta Paediatr Scand* 67 49 1973
 - 18 Orzalesi M Natoli G Panero A & Ciocca M Plasma hepatic enzymes in jaundiced newborn infants treated with phototherapy *In* D Bergsma & S H Blondheim (eds.) *Bilirubin Metabolism in the Newborn II Birth Defects Original Article Series* vol 12 no 2 Williams & Wilkins Baltimore 1976 p 91
 - 19 Rubaltelli F F Allegri G Costa C & De Antoni A Urinary excretion of tryptophan metabolites during phototherapy *J Pediatr* 91 865 1974
 - 20 Santella R M Rosenkranz H E & Speck W T Intracellular deoxyribonucleic acid modifying activity of intermittent phototherapy *J Pediatr* 93 106 1978
 - 21 Seligman J W Recent and changing concepts of hyperbilirubinemia and its management in the newborn *J Pediatr Clin North Am* 24 509 1977
 - 22 Smiles O R C Effect of phototherapy on thermal environment of the newborn *Arch Dis Child* 53 172 1978
 - 23 Speck W T Chen C C & Rosenkranz H S *In vitro* studies of effects of light and riboflavin on DNA and HeLa cells *Pediatr Res* 9 150 1975
 - 24 Tan K L Chow M T & Kanin S M M Effect of phototherapy on neonatal riboflavin status *J Pediatr* 93 494 1978

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A LONGITUDINAL STUDY OF MANGANESE IN HUMAN MILK

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ABSTRACT Vuori E (Department of Public Health Science University of Helsinki Helsinki Finland) A longitudinal study of manganese in human milk. *Acta Paediatr Scand* 68 571 1979.—Flameless atomic absorption spectrophotometry has been applied to the analysis of manganese in human milk samples. Twenty seven breast feeding mothers donated 29 individual milk samples between the 2nd week and the 9th month of lactation. The milk samples were representative of every feed in a period of 24 hours: foremilk and hindmilk in equal proportions. The median concentration of manganese declined from the initial value of $5.9 \mu\text{g/l}$ to about $4 \mu\text{g/l}$ before the 2nd month of lactation, remained at this level up to the 5th–6th month of lactation and showed a tendency to rise thereafter. The values presented here are noticeably lower than most of those reported earlier. It remains an open question whether the manganese concentration is exceptionally low in Finnish human milk or whether the great difference from most earlier studies may reflect problems of contamination or technical difficulties when less sensitive analytical methods have been used.

Key words: milk, human, manganese.

Compared with other trace elements such as iron, copper and zinc, the number of studies concerning the manganese concentration of human milk is small. The mean manganese concentration has ranged from 7 to $120 \mu\text{g/l}$ (2–5–7). Any variation to this extent in the average value of an essential trace element is not likely to be a physiological phenomenon. So far, no data is available to illustrate how the manganese concentration of human milk varies with the stage of lactation. The discrepant values on the manganese levels in human milk are also reflected in the recommendation for manganese in different milk formulas. In 1971, The Codex committee recommended the minimum manganese concentration in complete infant formula to be $200 \mu\text{g}/100 \text{ kcal}$, but in 1976 this figure was changed to $5 \mu\text{g}/100 \text{ kcal}$ (1, 2). More detailed data are obviously needed on the manganese concentration of human milk in order to achieve unanimity in this question.

SUBJECTS AND METHODS

Twenty seven breast feeding mothers supplied 29 milk samples between the 2nd week and the 9th month of

lactation. At first the mothers delivered samples at 2 week intervals and after the 2nd month at intervals of 3–4 weeks. Each individual milk sample was representative of every feed during a period of 4 hours and consisted of equal volumes of foremilk and hindmilk (10). The present method of collecting milk samples allowed the mother to continue breast feeding without interruption and minimized the potential diurnal variation and any variation in the composition of the milk within a single feed. The number of breast feeding mothers declined during the follow-up being 23, 13 and 7 in the 3rd, 6th and 9th month of lactation, respectively. The group of mothers and the method of collecting milk samples have been discussed earlier in detail (10).

After dry ashing at 450°C (10) the manganese concentration was determined from ash solutions with flameless atomic absorption spectrophotometry (Perkin Elmer model 300 with HGA-100) using deuterium background correction. Since it has been shown that calcium and magnesium can interfere with manganese determinations when the flameless technique is used (9), the influence of the major cations in concentrations present in mature human milk (4) on manganese standards was tested, but no effect was found. The accuracy of the method was studied by analysing Bovine Liver Standard Reference material No. 1577, National Bureau of Standards (NBS), Washington, D.C. Our mean result from four determinations was $11.0 \mu\text{g/g}$ dry weight, with a range of 10.8 – $11.7 \mu\text{g/g}$. The certified value for manganese is $10.3 \pm 1.0 \mu\text{g/g}$ as given by NBS. The precision of the method was established by repeated analysis of a pooled human milk sample. The mean results of 11 determinations was $6.4 \mu\text{g/l}$ ± 0.5 between series.

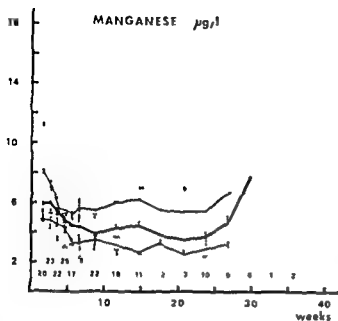


Fig. 1 The average manganese concentration of human milk during lactation showing the total range, median values and the 25th and the 75th percentiles after the 2nd week at weekly intervals and after the 7th week, thrice weekly. Total number of samples is 229. Numerals indicate the number of samples at each interval of time.

RESULTS AND DISCUSSION

The average values are expressed here by medians and the variation by giving the total range, the 25th and 75th percentiles since mean values and standard deviations overestimate the average concentration and variation due to the skewed distribution of values (Fig. 1). The median manganese concentration was 5.9 µg/l in the 2nd week of lactation and 50% of the values ranged between 4.9 and 7.0 µg/l. The median value declined to about 4 µg/l before the 2nd month of lactation and

remained at that level up to the 5th–6th month of lactation where after it showed a tendency to increase (Fig. 1). The lowest and highest individual values for manganese in human milk were 1.8 and 17.8 µg/l respectively.

Fomon's textbook on pediatric nutrition gives values of 7–15 µg/l as the normal level of manganese in mature human milk (4). The figures given are based on studies where only a few milk samples had been studied and the samples represent only the early stages of lactation (Table 1). The present results are noticeably lower than most of those reported earlier but are comparable with the results of the oldest study presented in Table 1.

When the effect of the stage of lactation on the manganese concentration of breast milk was studied it was found that manganese initially showed high values, then decreased to a minimum level and finally showed a rising tendency during the last months of the study. This pattern differs from that found in the case of zinc where decreasing values were noted throughout the course of lactation (10) and in the cases of copper and iron where a minimum level was found after the 4th–5th month of lactation (8, 10).

It remains an open question whether the manganese concentration is exceptionally low in Finnish human milk or whether the great difference from most earlier studies may reflect contamination problems or technical difficulties when less sensitive analytical methods have been used.

Table 1 The average manganese concentration of human milk according to different authors

Mn (µg/l)	No. of samples	Stage of lactation	Reference
7	Several	?	Broek & Wolff 1935 (3)
19	30	6th–7th day	Grebennikov et al. 1961 (5)
120	22	?	Murthy & Rhea 1971 (7)
15	10	after 1st week	McLeod & Robinson 1972 (6)
5.9 ^a	229	2nd week	Present study
7.8 ^a		31st week	

The authors give ppm in ash

^a Median value

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REFERENCES

- 1 ALINORM 76/ 6 Appendix IV 1971
- 2 ALINORM 76/ 6 Appendix III 1976
- 3 Broek A & Wolff L K The quantity of Cu Zn and Mn in cow's milk *Acta Brevia Neer Physiol Pharmacol Microbiol* 5 100 1975
- 4 Fomon S J *Infant Nutrition* W B Saunders Company Philadelphia 1974 2nd edn
- 5 Grebennikov E P Soroka V R & Sabadash E V Trace element in human and animal milk *Fed Proc* 23 461 1964
- 6 MLeod B E & Robinson M F Dietary intake of manganese by New Zealand infants during the first six months of life *Br J Nutr* 77 779 1977
- 7 Murthy G K & Rhea U M Cadmium copper iron lead manganese and zinc in evaporated milk infant products and human milk *J Dairy Sci* 54 1001 1971
- 8 Sumes M A Vuori E & Kuitunen P Breast milk iron - a declining concentration during the course of lactation *Acta Paediatr Scand* 68 29 1979
- 9 Smeijers Verbeke J Michotte Y Van den Winkel P & Massart D Matrix effects in the determination of copper and manganese in biological materials using carbon furnace atomic absorption spectrometry *Anal Chem* 48 175 1976
- 10 Vuori E & Kuitunen P The concentrations of copper and zinc in human milk *Acta Paediatr Scand* 68 33 1979

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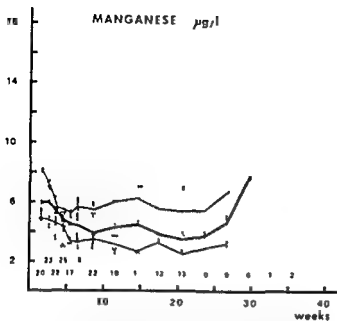


Fig. 1. The average manganese concentration of human milk during lactation, showing the total range, median values and the 25th and the 75th percentiles after the 2nd week at weekly intervals and after the 7th week three weekly. Total number of samples is 229. Numbers indicate the number of samples at each interval of time.

RESULTS AND DISCUSSION

The average values are expressed here by medians and the variation by giving the total range, the 25th and 75th percentiles since mean values and standard deviations over estimate the average concentration and variation due to the skewed distribution of values (Fig. 1). The median manganese concentration was 5.9 µg/l in the 2nd week of lactation and 50% of the values ranged between 4.9 and 7.0 µg/l. The median value declined to about 4 µg/l before the 2nd month of lactation and

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When the effect of the stage of lactation on the manganese concentration of breast milk was studied, it was found that manganese initially showed high values, then decreased to a minimum level and finally showed a rising tendency during the last months of the study. This pattern differs from that found in the case of zinc, where decreasing values were noted throughout the course of lactation (10) and in the cases of copper and iron, where a minimum level was found after the 4th–5th month of lactation (8, 10).

It remains an open question whether the manganese concentration is exceptionally low in Finnish human milk or whether the great difference from most earlier studies may reflect contamination problems or technical difficulties when less sensitive analytical methods have been used.

Table 1. The average manganese concentration of human milk according to different authors

Mn (µg/l)	No. of samples	Stage of lactation	Reference
7	Several	?	Broek & Wolff 1935 (3)
19	30	6th–7th day	Grebennikov et al. 1961 (5)
120	22	?	Murthy & Rhea 1971 (7)
15	10	after 1st week	McLeod & Robinson 1972 (6)
5.9 ^a		2nd week	
7.8 ^b	229	31st week	Present study

^a The authors give ppm in ash
^b Median value

UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

I Thrombosis in Relation to Catheter Type and Position

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ABSTRACT Westström G, Finnström O and Stenport G (Departments of Paediatrics and Diagnostic Radiology, University Hospital, Linköping, Sweden). Umbilical artery catheterization in newborns. I. Thrombosis in relation to catheter type and position. *Acta Paediatr Scand* 68:575, 1979. Seventy-one sick newborn infants who had an umbilical artery catheterized were randomized in one of four catheter groups: long end-hole, short end-hole, long side-hole or short side-hole catheter. A long catheter means a high position of the catheter tip (Th₁) and a short catheter a low position of the tip (L₁). An angiography through the indwelling catheter in order to diagnose thrombosis was performed before the catheter was withdrawn. Dissection of the aorta and its branches was performed on infants who died. The total frequency of thromboses was 26%. There were no thromboses among infants with long end-hole catheters while infants with short end-hole catheters had thrombosis in 26%, long side-hole catheters in 33% and short side-hole catheters in 64%. Long end-hole catheters functioned better than the others. Only 6 of 16 infants with thrombosis had physical signs from the legs while 12 infants without thrombosis had similar signs.

KEY WORDS Newborn infant, umbilical artery catheterization, thrombosis, aortography.

The use of prolonged umbilical vessel catheterization for the diagnosis and treatment of sick newborns is routine in most paediatric departments. In Sweden today roughly 1-6% of all newborn infants have an umbilical artery catheter introduced. The figure is based on a nationwide survey (7). Critically ill infants who are receiving oxygen therapy need careful monitoring of arterial oxygen tension (P_{O_2}) to allow adequate oxygenation and to reduce the threat of retrolental fibroplasia. Arterialized capillary blood is not suitable (14). Other procedures to obtain arterial blood than from the umbilical artery have been recommended but are less convenient. Therefore at present catheterization of the umbilical artery remains the standard method for obtaining arterial blood. However a variety of complications arising from this procedure has been described. Complications previously reported have included embolism, thrombosis, vascular perforation, ischemic necrosis of abdominal

viscera, infection, hemorrhage and blanching of the legs (2, 5, 9, 15, 17, 23).

The most common serious complication has been thrombosis. The rate varies widely in previously reported retrospective (5, 9, 16, 33) and prospective investigations (Table 1). The varying results have led to different recommendations to decrease the risks with regard to the type of catheter, the position of the catheter tip as well as the technique for catheterization (13, 19, 20).

The present prospective study was aimed at elucidating the following questions:

- the frequency of thrombosis associated with arterial umbilical catheterization
- the frequency of thrombosis with two different types of catheters and two different positions of the catheter tip
- the most suitable type and position of the catheter for good function
- the correlation between physical signs and thrombosis

Table 3 The occurrence of thrombosis in relation to type and position of the umbilical artery catheter

In 7 infants both angiography and autopsy were performed

Type and position of catheter	Infants (n)	Infants with angiography (n)	Thrombosis at angiography (n)	Infants with autopsy (n)	Thrombosis at autopsy (n)	Total number of thromboses		Drop outs (n)
						n	c	
Long end hole	0	14	0	6	0	0	0	2
Short end hole	14	15	3	5	2	5	26.3	4
Long side hole	12	11	4	1	0	4	33.3	1
Short side hole	11	11	7	1	0	7	63.6	1
Total	38	51	14	13	2	16	25.8	9

was registered as well as clinical signs that could be attributed to the catheterization.

The investigation was approved by the ethical committee of the medical faculty of the University of Linköping.

RESULTS

The distribution of the 62 infants as well as the number of thromboses within the four groups is shown in Table 3. Sixteen infants (26%) had thrombosis or embolus. Fourteen were diagnosed at angiography and 2 at autopsy. Thrombosis was present in all sub groups except LE catheter. The difference in number of thromboses between long and short catheters (4 and 12) was significant ($p < 0.05$) as well as the difference between end and side hole catheters (5 and 11) ($p < 0.01$). There was also a significant difference between LE and LS catheters ($p < 0.05$) and between LE and SE catheters ($p < 0.05$). The most pronounced difference ($p < 0.001$) was found between LE

and SS catheters. There was some difference though not significant between these four groups in the duration of catheterization as calculated from the introduction of the catheter until angiography (Table 4). Including the infants who died in whom the duration of catheterization was calculated until the moment the infant died there was significantly ($p < 0.05$) longer duration for side hole than for end hole catheters. The difference is due to the fact that more infants died in the end hole catheter groups. 11 in all. Most of these infants died early, often during the first day.

The children with and without thrombosis were compared with regard to several parameters (Table 5). There were no significant differences for gestational age, birth weight, Apgar score, degree of illness, duration of catheterization until angiography or the infusion of bicarbonate. Including the infants who died there was significantly ($p < 0.01$)

Table 4 Catheter type and clinical data: comparison between the four groups

Number of infants within parenthesis refer to duration of catheterization until angiography. Mean \pm SD

Type and position of catheter	No. of infants	Birth weight (g)	Gestational age (weeks)	Apgar score (5 min)	Degree of illness	Duration of catheterization until angiography (hours)	No. of infants receiving Sodium bicarbonate	Blood transfusion
Long end hole	0 (14)	8 \pm 967	35.8 \pm 3.1	7.7 \pm 0.5	2.9 \pm 1.5	61 \pm 7	8	3
Short end hole	19 (15)	2060 \pm 757	33.7 \pm 3.7	8.0 \pm 0.0	2.8 \pm 1.3	58 \pm 3	11	4
Long side hole	12 (11)	2090 \pm 925	34.7 \pm 3.2	8.0 \pm 1.8	6 \pm 1.0	64 \pm 44	6	3
Short side hole	11 (11)	2046 \pm 701	34.1 \pm 4.5	7.7 \pm 0.3	6 \pm 0.5	88 \pm 47	6	3

Table 1 Previous investigations in which the frequency of thrombosis at umbilical artery catheterization has been examined by angiography and autopsy

Author	Type and position of catheter	Angiography			Autopsy	
		n	Thrombosis (%)	Method	n	Thrombosis (%)
Neal et al 1972 (20)	Long end hole	18	95	Cine	12	48
Striuss et al 1974 (24)	Short end hole	24	90	Cine		
Olinsky et al 1975 (21)	Long and short end hole	30	30	Several pictures	8	63
Goetzman et al 1975 (8)	Long end hole	98	24	One picture	47	21
Mokrohnisky et al 1978 (19)	Long and short end hole	23	91	One picture		

MATERIAL

An attempt to catheterize an umbilical artery was performed on children fulfilling one or more of the clinical criteria given in Table 2. The catheterization was successful in 71 of 88 children (81%). The infants were randomized into 4 groups according to catheter type and localisation of the tip: Long end hole (The 11) (LE), short end hole (L₁ 3) (SE), long side hole (LS) and short side hole (SS) catheters.

These four groups were studied in parallel during a 15 months period. During the next four months the infants were separated in two groups only: short and long end hole catheters.

There were 9 drop-outs among the 71 children (Table 3) 4 because it was not possible to evaluate the angiography 3 due to an occlusion of the catheter without indication for recatheterization and 2 for other reasons. The remaining 62 infants are also shown in Table 3. Thirteen of these died: none of them because of catheter complications. There were no statistically significant differences between the four groups with regard to gestational age, birth weight, Apgar score, duration of catheterization until angiography, bicarbonate infusion, blood transfusion through the catheter and degree of illness (Table 4). Degree of illness was estimated according to a 5 degree scale, where score 5 consisted of the infants who died, score 4 and 3 the infants who needed mechanical ventila-

tion and continuous positive pressure (CPAP) respectively and score 2 and 1 the remaining less sick infants. Four infants received total parenteral nutrition (Intralipid and Vamine®) via the umbilical artery catheter.

METHODS

The catheterizations were done under strict aseptic conditions with a technique comparable to that recommended by Kitterman et al (13). Argyle umbilical artery catheter with end hole or Argyle feeding tube with side hole both made of polyvinyl chloride (IVC) were used. French size no 5 was the common size except in 5 infants where French size 3.5 was used. A high position of the catheter tip (The 11) was reached by introducing the catheter 1/3 a third of the child's body length (30) and a low position (L₁ 3) by using the body length and the figures given by Dunn (6). The position of the catheter was controlled by X-ray and adjusted if necessary. A 10% glucose solution was infused through the catheter. If necessary blood transfusion or other solutions including sodium bicarbonate were given through the catheter. The catheter was flushed with saline when withdrawing a blood sample or at least every 6th hour. A new catheter was inserted if it was no longer possible to withdraw a blood sample and there was still a need for blood samples. Heparin was not given. The catheter was withdrawn as soon as the clinical condition permitted. Before withdrawal angiography was performed by injecting manually 2-5 ml 45% Urografin® through the indwelling catheter. A cine film recording was performed in the first 33 infants, but thereafter one picture angiography was used. Thrombosis/embolism was considered to be present when at angiography an occlusion of the great pelvic or leg arteries was seen when filling defects in the aorta or pelvic arteries were seen or when there was an obvious sheath around the catheter.

At autopsy the aorta and all its major branches were dissected. The catheter was left in situ until autopsy.

The function of the catheter at aspiration and infusion

Table 2 Indications for umbilical artery catheterization

1. Birth weight below 1800 g
2. Gestational age 34 weeks or less
3. Postnatal asphyxia: Apgar score below 7 at 5 min or no spontaneous breathing after 5 min
4. Breathing difficulties: Silverman score more than 3 at 1 hour's age or clinical deterioration
5. Pronounced intrauterine growth retardation
6. Other severe neonatal diseases

Table 3 The occurrence of thrombosis in relation to type and position of the umbilical artery catheter

In infants both angiography and autopsy were performed

Type and position of catheter	Infants (n)	Infants with angiography (n)	Thrombosis at angiography (n)	Infants with autopsy (n)	Thrombosis at autopsy (n)	Total number of thrombosis		Drop outs (n)
						n	%	
Long end hole	20	14	0	6	0	0	0	0
Short end hole	19	15	3	5	2	5	26.3	4
Long side hole	17	11	4	1	0	4	33.3	1
Short side hole	11	11	7	1	0	7	63.6	0
Total	67	51	14	13	2	16	25.8	9

was registered as well as clinical signs that could be attributed to the catheterization

The investigation was approved by the ethical committee of the medical faculty of the University of Linköping

RESULTS

The distribution of the 62 infants as well as the number of thromboses within the four groups is shown in Table 3. Sixteen infants (26%) had thrombosis or embolus. Fourteen were diagnosed at angiography and 2 at autopsy. Thrombosis was present in all sub groups except LE catheter. The difference in number of thromboses between long and short catheters (4 and 12) was significant ($p < 0.05$) as well as the difference between end and side hole catheters (5 and 11) ($p < 0.01$). There was also a significant difference between LE and LS catheters ($p < 0.05$) and between LE and SE catheters ($p < 0.05$). The most pronounced difference ($p < 0.001$) was found between LE

and SS catheters. There was some difference though not significant between these four groups in the duration of catheterization as calculated from the introduction of the catheter until angiography (Table 4). Including the infants who died in whom the duration of catheterization was calculated until the moment the infant died there was significantly ($p < 0.05$) longer duration for side hole than for end hole catheters. The difference is due to the fact that more infants died in the end hole catheter groups: 11 in all. Most of these infants died early, often during the first day.

The children with and without thrombosis were compared with regard to several parameters (Table 5). There were no significant differences for gestational age, birth weight, Apgar score, degree of illness, duration of catheterization until angiography or the infusion of bicarbonate. Including the infants who died there was significantly ($p < 0.01$)

Table 4 Catheter type and clinical data: comparison between the four groups

Number of infants within parenthesis refer to duration of catheterization until angiography. Mean \pm S.D.

Type and position of catheter	No of infants	Birth weight (g)	Gestational age (weeks)	Apgar score (5 min)	Degree of illness	Duration of catheterization until angiography (hours)	No. of infants receiving	
							Sodium bicarbonate	Blood transfusion
Long end hole	0 (14)	2787 \pm 907	35.8 \pm 3.1	7.2 \pm 2.5	7.9 \pm 1.5	61 \pm 7	8	3
Short end hole	19 (15)	2060 \pm 757	33.7 \pm 3.7	8.0 \pm 2.0	7.8 \pm 1.3	58 \pm 3	11	4
Long side hole	1 (11)	090 \pm 975	34.7 \pm 3.7	8.0 \pm 1.8	7.6 \pm 1.0	64 \pm 44	6	3
Short side hole	11 (11)	2046 \pm 701	34.1 \pm 4.5	7.7 \pm 3	7.6 \pm 0.5	88 \pm 47	6	3

Table 5 Comparison between infants with and without thrombosis with regard to clinical data

Number of infants within parenthesis refers to duration of catheterization until angiography. Mean \pm S.D.

	No. of infants	Birth weight (g)	Gestational age (weeks)	Apgar score (5 min)	Degree of illness	Duration of catheterization until angiography (hours)	No. of infants receiving	
							Sodium bicarbonate	Blood transfusion
Infants without thrombosis	46 (37)	2 096 \pm 891	34 2 \pm 3.7	7.5 \pm 2.2	2.7 \pm 1.3	61 \pm 32	70	7
Infants with thrombosis	16 (14)	2 249 \pm 555	35.6 \pm 3.1	7.9 \pm 2.2	2.9 \pm 1.0	82 \pm 43	11	6

longer duration of catheterization for the infants with than without thrombosis.

Blood transfusions were more often given to children with thrombosis than without: 38 and 15% respectively. Three of the 4 infants who received total parenteral nutrition via the umbilical catheter had thrombosis. Exclusion of these 4 infants from the total material does not change the significant differences between the 4 catheter groups. There was no significant difference between the groups with regard to hemoglobin, hematocrit, activated partial thromboplastin time (APTT) or number of platelets.

No case of septicemia was found but blood and catheter cultures were positive in 13 and 28% respectively. The infants who developed thrombosis did not show an increased number of positive cultures. For further details regarding these aspects see Westrom et al. (31).

Table 6 Catheter problems at aspiration or infusion and recatheterization

Type and position of catheter	No. of infants	No. of infants with catheter problems	No. of infants recatheterized
Long end hole	22	2	~
Short end hole	23	11	5
Long side hole	13	3	2
Short side hole	13	11	6
Total	71	29	13

Three of the infants with thrombosis had a complete occlusion of the common iliac artery on the side of the catheterization. All these 3 infants had SS catheters. One infant with a SE catheter had an embolus which totally occluded the popliteal artery on the side opposite to that of the catheterization.

Colour changes of the legs were noted in 18 infants. The duration of these was short as a rule. Six of these infants had thrombosis or embolism. Thus ten infants with thrombosis lacked such signs.

The function of the catheters at aspiration and infusion was investigated. There were difficulties mainly with the short catheters and particularly with the SS catheters (Table 6). In three infants with SE catheters the catheter slipped out spontaneously in spite of fixation with a pursestring suture. There was no bleeding. For 69% of the infants with thrombosis there were problems with the catheter. The corresponding figure for infants without thrombosis was 28%.

DISCUSSION

The use of umbilical artery catheters in the care of sick newborn infants is a widely accepted method for getting samples for blood gas analysis. Other methods for receiving arterial blood such as repeated puncture or catheterization of the temporal or radial artery have limitations (1, 4, 27, 29). Transcutaneous P_{O_2} monitoring will probably reduce but not

replace the need of umbilical artery catheters in the future (12, 18, 25).

Umbilical artery catheterization involves certain risks. The most serious complication has been thrombosis or embolism. The frequency has varied considerably from 3.5 to 48% in postmortem investigations (5, 9, 16, 33) and from 24 to 95% in earlier angiographic investigations (Table 1). Different types of catheters and positions of the tips have been used. In the Swedish inquiry it was found that all four catheter types and positions that we studied were in use (7).

The present investigation showed a frequency of thromboses of 26%. The result is difficult to compare with previous investigations as the frequency varies with the type and position of the catheter (Table 3). Neal et al. (20) as well as Goetzman et al. (8) used LE catheters and found a frequency of thromboses of 95 and 24% respectively to be compared with 0% for the same type and position of the catheter in our study. Strauss et al. (24) used SE catheters and found thrombosis in 90% compared to 26% in the present investigation and 30% in the mixed (LE + SE) catheter material of Olinsky et al. (21). The great discrepancy between these results and ours is difficult to explain. Olinsky et al. (21) showed that a catheter filled with contrast medium and X-rayed in a contrast solution shows a translucence around the catheter. Translucence can easily be mistaken for thrombosis. This finding might explain some very high figures for thrombosis given in early investigations.

A possible advantage of side hole catheters is the smooth tip which theoretically may lessen the risk for damage of the vessel intima and thus for thrombosis. The present study showed quite the contrary. A reason might be the dead space in the tip of the side hole catheter where coagulation might occur leading to thrombosis.

An explanation for the difference in thrombosis between long and short catheters is more difficult to find. In our study we failed

to find a difference in fibrinolytic activity between different parts of the aorta (11). Another explanation could be a difference in blood velocity between the thoracic and the abdominal aorta. The blood flow in the thoracic aorta is greater than in the abdominal aorta since a great part of the blood leaves for the internal organs. The angiographic films showed that the abdominal aorta was only slightly smaller than the thoracic aorta. Thus the blood velocity in the abdominal aorta is probably slower which might lead to an increased risk for developing thrombosis.

Total parenteral nutrition via the umbilical artery catheter probably increases the risk for developing thrombosis since 3 of 4 who received parenteral nutrition had thrombosis.

A long duration of the catheterizations has been said to enhance the risk for thrombosis (26). Goetzman et al. (8) performed repeated angiographies on some infants and showed that when the first examination was negative the others were negative as well. In the present study there was a difference in the duration of the catheterization between infants with and without thrombosis. Therefore we think that the duration of the catheterization is at least of some importance for the occurrence of thrombosis.

Materials other than PVC in the catheters have been tried. Boros et al. (3) compared PVC and Silicone Elastomere (Silastic) catheters in a study of 20 infants. They found 90 and 10% thromboses respectively in the two non-randomized groups. The Silastic catheter is considerably softer and probably more difficult to handle and when used by the same authors in animal experiments thromboses were found in all cases at autopsy.

The present investigation as well as several earlier studies (9, 16, 20) have shown a poor correlation between clinical signs and thrombosis. As a rule therefore it is not possible to diagnose thrombosis from clinical signs only.

The function of the catheters is of great importance. A poorly functioning catheter

often has to be exchanged which may enhance the risk of complications. As shown in the results (Table 6) the function of the catheters varied considerably with different positions.

Angiography through the indwelling catheter is a safe method for diagnosing thrombosis in the aorta and its major branches. No complications related to the angiography were noted in this study nor in earlier reports. In the first part of this study cine angiography was used later on replaced by one picture angiography. The reason for this change was that the technically successful one picture angiography gave the same degree of information with a lower radiation dose and in a more simple way. The diagnosis of thrombosis by angiography may be difficult and under- and overestimation of thrombosis may occur. In order to overcome this problem two experienced roentgenologists evaluated all doubtful films in the present study. In 12 of 14 cases (Table 3) the diagnosis of thrombosis was regarded as without doubt. In the remaining 2 cases the diagnosis was probable. In one of them there were only sleeve like changes around the tip of the catheter and in the other there was a difference in blood pressure between the two legs, a finding that we have seen in another study (32) to be related to arterial thrombosis. Therefore the diagnosis based on angiography was probably correct.

Most authors recommend a low position of the catheter tip (13, 20, 21, 28). The present investigation showed however that the risk of thrombosis is significantly lower with a high position in which the catheters also function better. In two recent papers clinical signs of complications have been related to the position of the catheter tip (10, 19). Both groups arrived at the same conclusion as we did, i.e. a higher complication rate with a low catheter position. Thrombosis occurring with a high position of the catheter tip might theoretically lead to more serious consequences as the thrombosis may then affect the mesenteric or renal arteries (20, 21, 28). This is the main

reason why most authors recommend a low catheter position with the tip below the level of the main vessels to the internal organs. However these main vessels can be affected by thrombosis even when a short catheter is used perhaps from retrograde movement of clots (19). Furthermore a satisfactory low position is not always easy to obtain not even with fluoroscopy as the level at which the main vessels take off from the aorta varies (21, 22, 30). When using a high position of the catheter such problems do not exist.

In conclusion we believe that there is substantial evidence to recommend end hole catheters in a high position (Th₁₀₋₁₁) when performing umbilical artery catheterization in the newborn infant.

ACKNOWLEDGEMENT

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REFERENCES

- Adams J M & Rudolph A J. The use of indwelling radial artery catheters in neonates. *Pediatrics* 45: 261 1975.
- Andersson A, Hofer P Å, Holmlund D E W & Wendel H Å. Colonic perforation secondary to thrombo-embolus from an umbilical artery catheter. *Acta Paediatr Scand* 63: 155 1974.
- Boros S J, Thomson T R, Reynolds J W, Jarvis C W & Williams H J. Reduced thrombus formations with silicone elastomere (silastic) umbilical artery catheters. *Pediatrics* 56: 981 1975.
- Bucci G, Scalapandre A, Savignoni P G, Orzalessi M & Mendicino M. Crib-side sampling of blood from the radial artery. *Pediatrics* 37: 497 1966.
- Cochran W D, Davis H T & Smith C A. Advances and complications of umbilical artery catheterization in the newborn. *Pediatrics* 47: 769 1968.
- Dunn P M. Localization of the umbilical catheter by postmortem measurement. *Arch Dis Child* 41: 69 1966.
- Finnstrom O, Stenport G & Westrom G. Catheterization of the umbilical artery in neonates. *Läkärstämman* 75: 2627 1978.
- Goetzman B W, Stadlnik R C, Bogen H G, Blankenship W J, Ikeda H M & Thayer J. Thrombotic complications of umbilical artery catheters. A clinical and radiographic study. *Pediatrics* 56: 374 1975.
- Gupta J M, Robertson N E C & Wigglesworth J S. Umbilical artery catheterization in the newborn. *Arch Dis Child* 43: 382 1968.

- 10 Harris M S & Little G A Umbilical artery catheters: High, low or no? *J Perinat Med* 6: 15 1978
- 11 Henriksson P, Westström G & Hedner U Umbilical artery catheterization in newborns. III. Thrombosis—A study of some predisposing factors. *Acta Paediatr Scand* in press
- 12 Huch E, Lubbers D W & Huch A Reliability of transcutaneous monitoring of arterial PO₂ in newborn infants. *Arch Dis Child* 49: 713 1974
- 13 Kitterman J A, Phibbs R H & Tooley W H Catheterization of umbilical vessels in newborn infants. *Pediatr Clin North Am* 17: 895 1970
- 14 Koch G & Wendel H Comparison of Ph carbon dioxide tension, standard bicarbonate and oxygen tension in capillary blood and in arterial blood during the neonatal period. *Acta Paediatr Scand* 56: 10 1967
- 15 Krauss A N, Albert R F & Kannan M M Contamination of umbilical catheters in the newborn infant. *J Pediatr* 77: 965 1970
- 16 Larroche J Cl Umbilical catheterization: Its complications. *Biol Neonate* 16: 101 1970
- 17 Livaditis A, Wallgren B & Faxälv G Necrotizing enterocolitis after catheterization of the umbilical vessels. *Acta Paediatr Scand* 63: 777 1974
- 18 Lofgren O, Henriksson P, Jacobson L & Johanson O Transcutaneous PO₂ monitoring in neonatal intensive care. *Acta Paediatr Scand* 67: 693 1978
- 19 Mokrohisky S T, Levine R L, Blumhagen J D, Wesenberg R L & Simmons M A Low positioning of umbilical artery catheters increases associated complications in newborn infants. *N Engl J Med* 290: 561 1978
- 20 Neal W A, Reynolds J W, Jarvis C W & Williams H J Umbilical artery catheterization: Demonstration of arterial thrombosis by aortography. *Pediatrics* 50: 6 197
- 21 Olinsky A, Aitken F G & Isdale J M Thrombus formation after umbilical arterial catheterization: An angiographic study. *S Afr Med J* 49: 1467 1975
- 22 Phelps D L, Lachman R S, Leake R D & Oh W The radiologic localization of the major aortic tributaries in the newborn infant. *J Pediatr* 81: 336 1972
- 23 Rajs J, Finnström O & Westström G Aortic aneurysm developing after umbilical artery catheterization. *Acta Paediatr Scand* 65: 495 1976
- 24 Strauss A W, Escobedo M & Goldring D Continuous monitoring of arterial oxygen tension in the newborn infant. *J Pediatr* 85: 254 1974
- 25 Swanström M & Rooth G The value of transcutaneous PO₂ measurements in a newborn infant in shock. *Paediatrician* 5: 304 1976
- 26 Symansky M R & Fox H A Umbilical vessel catheterization: Indications, management and evaluation of the technique. *J Pediatr* 80: 870 1972
- 27 Thomsen A Arterial blood sampling in small infants. *Acta Paediatr Scand* 53: 237 1964
- 28 Tooley W H What is the risk of an umbilical artery catheter? *Pediatrics* 50: 1 1977
- 29 Wall P M & Kuhns L R Percutaneous arterial sampling using transillumination. *Pediatrics* 59: 1037 1977
- 30 Weaver H L & Ahlgren E W Umbilical artery catheterization in neonates. *Am J Dis Child* 172: 499 1971
- 31 Westström G & Finnström O Umbilical artery catheterization in newborns. II. Infections in relation to catheterization. *Acta Paediatr Scand* in press
- 32 Westström G & Lassvik C To be published
- 33 Wigger H J, Bransilver B R & Blanc W A Thrombosis due to catheterization in infants and children. *J Pediatr* 76: 1 1970

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REFERENCES

- 1 Adams J M & Rudolph A J. The use of indwelling radial artery catheters in neonates. *Pediatrics* 55: 261 1975.
- 2 Andersson A, Hofer P Å, Holmlund D E W & Wendel H Å. Colonic perforation secondary to thromboembolus from an umbilical artery catheter. *Acta Paediatr Scand* 63: 155 1974.
- 3 Boros S J, Thomson T E, Reynolds J W, Jarvis C W & Williams H J. Reduced thrombus formations with silicone elastomere (silastic) umbilical artery catheters. *Pediatrics* 56: 981 1975.
- 4 Bucci G, Scalapandre A, Savignoni P G, Orzalesi M & Mendicino M. Crib-side sampling of blood from the radial artery. *Pediatrics* 37: 497 1966.
- 5 Cochran W D, Davis H T & Smith C A. Advantages and complications of umbilical artery catheterization in the newborn. *Pediatrics* 47: 769 1969.
- 6 Dunn P M. Localization of the umbilical catheter by postmortem measurement. *Arch Dis Child* 41: 69 1966.
- 7 Finnstrom O, Stenport G & Westrom G. Catheterization of the umbilical artery in neonates. *Läkartidningen* 75: 2627 1978.
- 8 Goetzin D W, Stridalm R C, Bogen H G, Blankenship W J, Ikeda R M & Thayer J. Thrombotic complications of umbilical artery catheters. A clinical and radiographic study. *Pediatrics* 56: 374 1975.
- 9 Gupta J M, Robertson N R C & Wigglesworth J S. Umbilical artery catheterization in the newborn. *Arch Dis Child* 43: 382 1968.

ADYNAMIA EPISODICA HEREDITARIA

Treatment with Salbutamol

K. DAHL JØRGENSEN and H. MICHALSEN

From the Department of Paediatrics Aker Hospital Oslo Norway

ABSTRACT Dahl Jørgensen K and Michalsen H (Department of Paediatrics Aker Hospital Oslo Norway) Adynamia episodica hereditaria Treatment with salbutamol *Acta Paediatr Scand* 68 583 1979 —Three sibs with adynamia episodica hereditaria are described Treatment with salbutamol inhalation had a beneficial effect on the duration of their adynamic attacks Continuous peroral treatment with salbutamol has been tried in these patients resulting in the almost complete prevention of attacks No serious adverse effects have been recorded Peroral treatment with salbutamol is recommended as the treatment of choice in young patients with adynamia episodica hereditaria and in patients with frequent attacks of adynamia

KEY WORDS Adynamia episodica hereditaria salbutamol treatment

Adynamia episodica hereditaria (AEH) a disease inherited as an autosomal dominant is characterized by early onset of short and frequent episodes of muscle weakness accompanied by increasing serum potassium (1) Drugs which are reported to shorten an attack when given during an attack are calcium intravenously catecholamines subcutaneously and inhalation of salbutamol (2-3-4) The effect of salbutamol *in vitro* has been shown to be related to the hypokalemic effect due to stimulation of the active Na and K transport in the muscle cells (5) Acetazolamid has for many years been used successfully to prevent attacks (1-2)

The diagnosis of AEH has been established in 3 siblings their mother being the only previously known case of AEH in Norway (2) The beneficial effect of salbutamol inhalation was confirmed in these patients Prophylactic treatment with salbutamol successfully reduced the frequency of the attacks

PATIENTS

The mother of our patients had early manifestations of AEH but her diagnosis was not confirmed until 10 years

ago The father of our patients has no sign of AEH The couple have 3 children all suffering from AEH

Patient 1 is a girl (13 years old) patients 2 and 3 are boys (9 and 7 years old) Their symptoms started before 1 year of age Their mother describes the symptoms as short periods of muscular hypotonia At the age of 17-14 months the children commenced to walk and from then on the muscular weakness has appeared in rather distinct attacks Intellectual and motor development is normal Patient 2 had an attack of supraventricular paroxysmal tachycardia 7 years old the children have otherwise not suffered from serious diseases No previous examinations have been performed

The pattern of attacks is identical in the 3 patients They have from 2 to 4 daily attacks each attack lasting 15-30 min During these episodes the children are unable to walk or maintain an upright position Nightly attacks occur on the average once a week in each patient These are more severe generally lasting for 2-3 hours and are regularly accompanied by the subjective experience of respiratory distress The parents confirm that during nightly attacks the respiration appears distressed audible respirations are present with both inspiratory and expiratory stridor Patient 1 has moderate signs of myotonia in addition to the attacks of adynamia No treatment has been tried prior to their admission in our department but the children had experienced that active or passive movements of the affected limbs did shorten the attacks

RESULTS

Clinical and neurological examination of the children was normal when performed in intervals between the attacks

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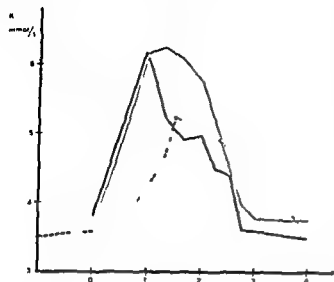


Fig. 1 Serum concentrations of potassium in the three patients during an attack.

Muscular weakness occurred spontaneously in patient 1 and 2. In patient 3 an episode was provoked during rest following 30 min of muscular exercise. During the attacks of adynamia we found flaccid weakness in the muscles of the face and extremities, the weakness being most pronounced in the lower limbs. The tendon reflexes were weaker than prior to the attack.

Elevated serum levels of potassium (Fig. 1) as well as hyperkalemic ECG changes were observed in all 3 patients during the attack. Electromyography performed between attacks is described as normal, the only possible abnormality being a moderate increase in spontaneous activity. Peripheral motor conduction rates were within normal limits. Assays of T_3 , T_4 and TSH did not reveal any signs of thyroid disorder. In patients 1 and 3 biopsies from the anterior tibial muscle were performed. Light microscopy and electron microscopy revealed no distinct abnormalities.

Inhalation of salbutamol alleviated hyperkalemia and clinical symptoms within few minutes. Patient 1 managed to use the inhalator according to instructions and was satisfied with the effect of this treatment. The two younger patients tried the inhalator during a later phase of the attacks and were therefore often unable to manage it effectively due to

Table 1 Number of adynamic attacks during 1 week without treatment (Period I) during 1 week with placebo (Period II) and during 1 week with salbutamol. Periods II and III were performed double blind, the tablets having identical appearance

	Period I	Period II	Period III
Patient 1	14	13	7
Patient 2	14	14	7
Patient 3	11	12	1

weakness of the upper limbs. However, the duration of their attacks were shortened by inhalations when they were helped by others. In some cases the inhalations had to be repeated once or twice in order to alleviate the symptoms.

We determined to try salbutamol tablets as a possible prophylactic treatment. Salbutamol was administered in doses of 0.3–0.4 mg/kg body weight divided in 4 daily doses. On this treatment the patients have been almost free from the episodes of adynamia. Patient 1 has continued, however, to have a moderate increase in the frequency of the myotonic episodes. No side effects have been noticed.

After more than 6 months on peroral salbutamol treatment we decided to perform a trial with placebo tablets having identical taste and appearance as the salbutamol tablets. The patients were without treatment for one week prior to the trial, each attack during this and the following weeks was carefully recorded by their mother. Placebo and salbutamol were given on alternating weeks, neither their mother nor their pediatrician knew which of the tablets the patients were given. The results of this trial are summarized in Table 1. The beneficial effect of prophylactic treatment is clearly demonstrated.

DISCUSSION

The diagnosis of these 3 patients is based on the typical history and the elevated serum

concentrations of potassium during the attacks of muscle weakness

Treatment with salbutamol inhalations was of questionable value in our patients due to the frequency and severity of their attacks. The two younger patients had trouble learning the technique of correct and early inhalation. After the initiation of peroral treatment the children have been virtually free from attacks. The observation period is presently 9 months. Trial with placebo tablets resulted in prompt recurrence of the attacks.

The doses of salbutamol presently used have had no detectable sympathomimetic effects. We are not aware of serious adverse reactions related to long term treatment with salbutamol. The patients do however demonstrate more myotonic attacks than prior to salbutamol therapy; this effect is most pronounced in the oldest patient. During the placebo experiment her myotonia almost disappeared; she did however have two daily attacks of adynamia during this period.

Acetazolamid has until now been the drug of choice in the prevention of weakness in AEH. This drug is known to have numerous though rare side effects: skin reactions, liver

damage, bone marrow depression, kidney lesions resembling those seen with sulpho namides and renal calculi are among the adverse reactions related to treatment with acetazolamid. Long term treatment with acetazolamid may thus be more risky than with salbutamol. Our patients have responded well to this apparently safe drug. We therefore recommend the use of peroral salbutamol treatment in young patients with AEH and in patients with frequent attacks.

REFERENCES

- 1 Gamstorp I: Adynamia episodica hereditaria. *Acta Paediatr Scand* 45 Suppl 108 1946
- 2 Hovstad L, Henriksen O & Try K: Adynamia episodica hereditaria. *Nord Med* 80 1065 1968
- 3 Klein R, Egan T & Usher P: Changes in sodium, potassium and water in hyperkalemic familial periodic paralysis. *Metabolism* 11 1005 1960
- 4 Wang P & Clausen T: Treatment of attacks in hyperkalemic familial periodic paralysis by inhalation of salbutamol. *Lancet* i 21 1976

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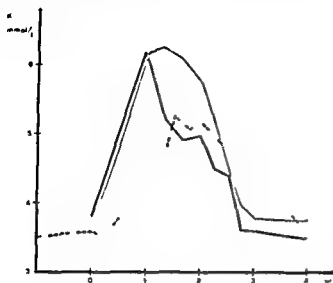


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MALE HYPOSPADIAS 625 CASES ASSOCIATED MALFORMATIONS AND POSSIBLE ETIOLOGICAL FACTORS

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ABSTRACT Svensson J (Department of Paediatric Surgery, Karolinska sjukhuset, Stockholm, Sweden). Male hypospadias 625 cases. Associated malformations and possible etiological factors. *Acta Paediatr Scand* 68: 587-1978. —Records from 625 patients with hypospadias have been reviewed with reference to associated malformations. Cryptorchidism was found to be the most common associated malformation and was present in 6% of cases with hypospadias. The highest incidence of cryptorchidism was found in the more severe forms of hypospadias. Other concomitant genital malformations such as bifid scrotum, hypoplasia of penis and remnants of the Mullerian system showed the same pattern. The recorded incidence of bifid scrotum and hypoplasia of the penis was 4% and 8% respectively. A high incidence of abnormalities in the lower urinary tract was found in 50% of 84 patients investigated. The hypothesis of hypospadias being a result of testicular hormone insufficiency is discussed.

KEY WORDS Hypospadias, cryptorchidism, lower urinary tract.

Hypospadias is one of the most common developmental malformations with an incidence in Sweden of one case per 350 male births (4). In most cases the etiology of the malformation is unknown.

Several authors have found a hereditary element in hypospadias. Sorensen (32) reports that about 10% of brothers of hypospadiac patients were affected. Chen et al (7) estimate the heritability according to Falconer (10) as 74.1% indicating a polygenic etiology in most cases and that hereditary factors represent 74.1% of the causation of the malformation. The risk of recurrence within one generation has been estimated as approximately 6% (23).

Male pseudohermaphrodites may be defined as individuals with testicular gonads whose external genitalia have not developed into the normal male appearance. In this broad sense all males with hypospadias are male pseudohermaphrodites although in the majority of cases the male sex of hypospadiac pa-

tients is not questioned. In the more severe forms of hypospadias doubts may be raised as to the sex role in which the patient should be reared.

In a cytogenetic study of 80 cases of hypospadias Aarskog (1) found six patients with abnormal karyotypes. Three with XX/XY, two with XO/XY mosaicism and one patient with a normal female karyotype; the latter was a case of congenital virilizing adrenal hyperplasia. All six had severe hypospadias of penile (one), penoscrotal (four) and scrotal (one) type. A high incidence of sex chromosome aberrations is found in patients with hypospadias and associated cryptorchidism (2, 26).

Some cases of male pseudohermaphroditism with hypospadias have been ascribed to errors in the biosynthesis of androgens (37) and defects in the peripheral action of androgens on the target cells in the genital region. The development of the external genitalia in the male is dependent on testosterone secreted

Table 3 Number of recorded malformations in the lower urinary tract in different types of hypospadias found at voiding cystourethrography

Type of hypospadias	No of patients examined	Vesico-ureteral reflux	Urethral folds		Urethral recesses
			Obstructive	Non obstructive	
Glandular	17	2	—	5	1
Distal penile	8	3	3	5	—
Mid penile	9	1	1	—	1
Proximal penile	6	—	2	1	1
Penoscrotal	16	—	—	7	3
Scrotal	4	—	—	—	3
Perineal	4	—	1	—	2
	84	6	7	20	11

scrotum in 22%. Farkas also found that the incidence of associated anomalies was highest in patients with severe forms of hypospadias.

The aim of this retrospective study was to review a series of patients with hypospadias and the presence of associated malformations mainly as a background for studies on the etiology of hypospadias and the late somatic and psychological sequelae of this genital abnormality.

CLINICAL MATERIAL

During the period 1951–1977, 675 boys were admitted to the Department of Paediatric Surgery, Karolinska sjukhuset for treatment of hypospadias. Excluded from this study are patients with insignificant lesions not considered to require admittance for surgical correction.

The different degrees of hypospadias included in the

survey are listed in Table 1. Operations (meatotomy, correction of chordee and urethroplasty) were carried out by several surgeons using techniques described by Sir Denis Browne (5) or by Crawford (8). Surgical treatment was not yet completed in all patients at the end of 1977. The median ages for meatotomy, correction of chordee and urethroplasty were 5.3, 9 and 5 years respectively.

ASSOCIATED MALFORMATIONS

All figures in this study are based on records from patients during a 21 year period. In this kind of retrospective study there is a certain risk of under-estimation of associated malformations, since these may not always have been properly recorded.

Cryptorchidism was recorded in 36 patients (6%) bilateral in 23 cases (4%). The scrotum was bifid in 2 patients (4%). Hypoplasia of the penis as judged subjectively by the surgeon was noted in 40 cases (8%). Like cryptorchidism and bifid scrotum, hypoplasia of the penis was found more often in the more severe forms of hypospadias (Table 1).

The lower urinary tract was examined by voiding cystourethrography in 111 patients (17%) and of these 40% had some form of abnormality (Table 3).

Vesicoureteric reflux was seen in 6 patients. Transverse fold formations in the anterior wall of the prostatic urethra just opposite to the verumontanum were observed in 5 patients. Of these folds, five were slightly obstructive. True obstructive posterior urethral valves were observed in two patients.

Recesses of various sizes in the posterior wall of the prostatic and bulbous urethra were visualized at voiding cystourethrography in 11 patients. It could not be determined whether these recesses represented a partially developed vagina or dilated ducts of the bulbo-urethral glands (?). However, in one patient with scrotal hypospadias the pouch continued directly into a uterus. Biopsies from the gonads of this patient showed both testicular and ovarian tissue. The urethral pouches were common in the more severe cases of hypospadias (Table 3).

Table 4 Associated malformations recorded in different types of hypospadias

Type of malformation	No of patients with the malformation	Type of hypospadias
Ventricular septal defect	1	Glandular
Deafness	1	Distal penile
VOC anal atresia	2	Glandular
		Penoscrotal
Hydrocephalus	1	Glandular
Omphalocele	1	Glandular
Esophageal atresia	1	Mid penile
Anal atresia	1	Mid penile

Table 1 Total number of patients with hypospadias included in the study and the different types of operations performed

n = number of patients

	Total number		Meatotomy only		Meatotomy plus other urethral surgery		Correction of chordee		Urethroplasty	
	n	%	n	%	n	%	n	%	n	%
Glandular	219	35	95	43	33	15	56	26	84	38
Distal penile	195	31	23	12	67	34	100	41	155	81
Mid penile	93	15	1	1	18	19	58	63	86	93
Proximal penile	43	7	-	-	11	26	32	74	39	91
Penoscrotal	47	7	-	-	3	7	37	88	36	88
Scrotal	11	2	-	-	1	9	10	91	10	91
Perineal	22	3	-	-	2	9	21	95	21	95
	625		119 (19%)		135 (15%)		314 (50%)		434 (69%)	

from the fetal testis (20). Testosterone is metabolized in the target cells of the external genital region by an enzyme 5 α reductase to 5 α dihydrotestosterone (5 α DHT) which is supposed to be the active intracellular androgen in this region (30-35). 5 α DHT is bound to a soluble cytoplasmic receptor of protein nature and the 5 α DHT protein complex is then transferred into the nucleus where it exerts its effects on the genome (24). A defective 5 α reduction has been described in cases of pseudovaginal perineoscrotal hypospadias a rare hereditary disease (33). Lack of androgen receptor as a cause of hypospadias in human males has also been described (14).

Exogenous factors such as progestins given to the mother during pregnancy have been postulated to be an etiological factor in some cases of hypospadias (1).

The incidence of other malformations associated with hypospadias is high 18-32% (11-36). Cryptorchidism was found to be the most commonly associated malformation recorded in 3-19% of the patients (6-9, 11, 28, 31, 34, 36). The incidence of cryptorchidism in boys of one year of age in the normal population has been estimated to about 0.7% (29). Farkas (12) reporting 505 cases of hypospadias found that cryptorchidism was present in 13%, hypoplasia of the penis in 35% and bifid

Table 2 Number of recorded associated malformations in the external genitalia in different types of hypospadias

n = number of patients r = Spearman's rank correlation coefficient

Type of hypospadias	Cryptorchidism		Bifid scrotum		Hypoplasia of penis	
	n	%	n	%	n	%
Glandular	7	3	1	0.5	8	4
Distal penile	7	4	-	-	5	3
Mid penile	4	4	1	1	8	6
Proximal penile	6	13	2	5	5	17
Penoscrotal	2	5	4	10	9	21
Scrotal	3	27	3	28	2	18
Perineal	7	33	11	50	15	68
	36 (6%)		22 (4%)		50 (8%)	
	(r = 0.96 p < 0.01)		(r = 0.85 p < 0.05)		(r = 0.93 p < 0.02)	

Table 3 Number of recorded malformations in the lower urinary tract in different types of hypospadias found at voiding, cystourethrography

Type of hypospadias	No of patients examined	Vcico ureteral reflux	Urethral folds		Urethral recesses
			Obstructive	Non obstructive	
Glandular	17	?	-	5	1
Distal penile	28	3	3	5	-
Mid penile	9	1	1	?	1
Proximal penile	8	-	?	1	1
Penoscrotal	16	-	-	7	3
Scrotal	4	-	-	-	3
Perineal	4	-	1	-	?
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Hydrocephalus	1	Penoscrotal
Omphalocele	1	Glandular
Esophageal atresia	1	Glandular
Anal atresia	1	Mid penile
		Mid penile

Table 5 Patients with hypospadias in which chromosome studies were performed

Type of hypospadias	Cryptorchidism	Chromosome constitution
Mid penile	No	46 XY
Mid penile	No	46 XY
Proximal penile	Bilateral	45 XO/46 XY
Penoscrotal	No	46 XY
Penoscrotal	Bilateral	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Scrotal	No	46 XY
Scrotal	Bilateral	46 XY
Scrotal	No	45 XO/46 XY/47 XYY

Other abnormalities noted in voiding cystourethrography were ureteral remnant (one), urethral diverticulum (one), absence of verumontanum (two), neurogenic bladder (one), and duplication of the ureters (one).

Intravenous pyelography was performed in only 10 patients, five of whom had slight hydronephrosis not requiring surgery.

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DISCUSSION

The differentiation of the fetus into an individual of male status starts early in embryonic life, when the indifferent gonad is developed into a testis at a gestational age of 7 weeks (20). The external genitalia start to differentiate at the same time as Leydig cells appear in the testis at the 8th week (20). The initiation of testosterone secretion from the Leydig cells is believed to be stimulated by chorionic gonadotrophin (hCG) from the maternal circulation. Later in fetal life, about the 12th week, the testosterone secretion is controlled

by LH from the fetal pituitary gland (21). The plasma and testicular testosterone concentrations rise simultaneously to reach a sharp peak at the 12th week (3, 25, 27). It is assumed that the development of the external genitalia in the male is dependent upon testosterone production from the testis (20). Testosterone is metabolized in the genital skin to 5 α dihydrotestosterone which is thought to be the active intracellular androgen in this area (30). The Mullerian structures in the male disappear from the 8th week under the influence of a peptide hormone, Mullerian inhibiting hormone (MIH), secreted by the fetal Sertoli cells (17, 18).

In the 14 week old fetus the penile corporal part of the urethra is fully developed with the meatus at the base of the glans. Later in the 4th gestational month, formation of the glandular portion of the urethra is completed from an intrusion of an epithelial cord on the ventral side of the glans (16) and the meatus is now in its normal position.

In hypospadias the development of the external genitalia is arrested before its full differentiation. Several of our patients had signs of testicular insufficiency. Bifid scrotum and hypoplasia of the penis could be explained by an insufficient secretion of androgens from the testis. The presence of remnants of a uterus and a vagina in the males indicates defective testicular activity on the development of the male genital system. In cases where remnants of uterus are present in males, a defective action of the Mullerian inhibiting hormone is probably the cause. Since the regression of the Mullerian structures and the development of the external genitalia are taking place at about the same time in the 60-day old male fetus, a connection between the hypospadias and the remnants of the Mullerian structures in the individuals described above is highly suggestive. The descent of the testes takes place during the last month of fetal life (29). We found a high incidence of cryptorchidism in this material, especially in patients with the more severe forms of hypospadias.

One may speculate on a common etiology between some cases of hypospadias and cryptorchidism ■■ an androgen deficiency affecting both the development of the external genitalia and the descent of the testis although during different stages of fetal development

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REFERENCES

- Aarhøg D. Clinical and cytogenetic studies in hypospadias. *Acta Paediatr Scand Suppl* 03 1970
- Aarhøg D. Intersex masquerading as simple hypospadias. *Br J Defects* 7 177 1971
- Abramovich D R & Rowe H. Foetal plasma testosterone levels at mid pregnancy and at term: relationship to foetal sex. *J Endocrinol* 56 671 1973
- Avellan L. The incidence of hypospadias in Sweden. *Scand J Plast Reconstr Surg* 9 179 1975
- Browne D. Hypospadias. *Postgrad Med J* 25 367 1949
- Cendron J. Treatment of hypospadias. *Proc R Soc Med* 64 15 1964
- Chen Y C & Woolley F V. Genetic studies in hypospadias in males. *J Med Genet* 8 353 1971
- Crawford H S. The reconstructive surgery of the urethra with special reference to hypospadias. *Ann R Coll Surg Engl* 41 37 1967
- Culp O S. Struggles and triumphs with hypospadias and associated anomalies: review of 400 cases. *J Urol* 96 339 1966
- Faloner D S. The inheritance of liability to certain diseases estimated from the incidence among relatives. *Ann Hum Genet* 29 51 1965
- Fallon B Devine F J & Horton C E. Congenital anomalies associated with hypospadias. *J Urol* 116 985 1976
- Farkas L G. Hypospadias. *Czech Publ Acad Sci Prague* 1967
- Fraccaro M, Lindsten J, Klinger H P, Tiepolo L, Bergstrand C C, Herrlin K M, Lavadini A, Peterson M & Tillinger K G. Cytogenetical and clinical investigations in four subjects with anomalies of sexual development. *Ann Hum Genet* 29 81 1964
- Giffin J E, Punyashitla K & Wilson J D. Comparison of cells from control subjects and from patients with hereditary male pseudohermaphroditism due to androgens resistance. *J Clin Invest* 57 134 1976
- Hadjilovom F. Cryptorchidism: Ultrastructure of normal and cryptorchid testis development. *Adv Anat Embryol Cell Biol* 53 3 1977
- Jirasek J A. Principles of reproductive embryology. In J L Simpson (ed.) *Disorders of sexual differentiation*. Academic Press Inc New York and London 1976 p 57
- Josso N. Permeability of membranes to the Mullerian-inhibiting substance synthesized by the human fetal testis *in vitro*. A clue to its biochemical nature. *J Clin Endocrinol Metab* 34 65 1972
- Josso N. Mullerian-inhibiting activity of human fetal testicular tissue deprived of germ cells by *in vitro* irradiation. *Pediatr Res* 8 755 1974
- Josso N. Development and descent of the fetal testis. In J H Buich (ed.) *Maldevelopment of testis*. Urban und Schwarzenberg München Wien and Baltimore 1977 p 3
- Jost A, Vigier B, Prépin J & Percheret J P. Studies on sex differentiation in mammals. *Recent Prog Horm Res* 29 1 1973
- Kaplan S L, Grumbach M M & Aubert M L. The ontogenesis of pituitary hormones and hypothalamic factors in the human fetus. Maturation of central nervous system. Regulation of anterior pituitary function. *Recent Prog Horm Res* 32 161 1976
- Kjellberg S E, Ericsson N O & Rudhe U. The 17 α -oestrogen effect in childhood. Some correlated clinical and endocrinologic observations. *Almqvist & Wiksell Stockholm* 1947 p 33
- Lowry R B & Kliman M R. Hypospadias in successive generations: possible dominant gene inheritance. *Clin Genet* 9 85 1976
- Mason-Waring W S P. *The mechanism of action of androgens*. Springer Verlag New York Heidelberg and Berlin 1976 p 46
- Payne A H & Jaffe R B. Androgen formation from pregnenolone sulfate by fetal neonatal prepubertal and adult human testes. *J Clin Endocrinol Metab* 40 107 1975
- Rayfer J & Walsh P C. The incidence of intersexuality in patients with hypospadias and cryptorchidism. *J Urol* 116 769 1976
- Reyes F J, Boroditsky R S, Winter J S D & Fauman C. Studies on human sexual development II. Fetal and maternal serum gonadotropin and sex steroid concentrations. *J Clin Endocrinol Metab* 38 61 1974
- Ross J F, Farmer A W & Lindsay W A. Hypospadias. A review of 730 cases. *Plast Reconstr Surg* 4 357 1959
- Scorer C G & Farrington G H. *Congenital deformities of the testis and epididymis*. Butterworths London 1971
- Sinen P E & Wald J D. Testosterone formation and metabolism during sexual differentiation in the human embryo. *J Clin Endocrinol Metab* 38 113 1974
- Sykes P J & Ho L C Y. Hypospadias. A report on 193 treated cases. *Plast Reconstr Surg* 50 457 1977
- Sørensen H R. *Hypospadias with special reference to etiology*. E Munksgaard Copenhagen 1953
- Walsh P C, Madden J P, Harrod M J, Gold

Table 5 Patients with hypospadias in which chromosome studies were performed

Type of hypospadias	Cryptorchidism	Chromosome constitution
Mid penile	No	46 XY
Mid penile	No	46 XY
Proximal penile	Bilateral	45 XO/46 XY
Penoscrotal	No	46 XY
Penoscrotal	Bilateral	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Penoscrotal	No	46 XY
Scrotal	No	46 XY
Scrotal	Bilateral	46 XY
Scrotal	No	45 XO/46 XY/47 XYY

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A PROSPECTIVE PSYCHOLOGICAL AND CYTOGENETIC STUDY OF THREE GIRLS WITH MOSAIC MONGOLISM

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From the Departments of Paediatrics and Child Psychiatry, Karolinska Institutet, St. Goran's Children's Hospital and the Department of Clinical Genetics, Karolinska Hospital, Stockholm, Sweden

ABSTRACT Linne T (Departments of Paediatrics and Child Psychiatry, Karolinska Institutet, St. Goran's Children's Hospital and Department of Clinical Genetics, Karolinska Hospital, Stockholm, Sweden) A prospective psychological and cytogenetic study of three girls with mosaic mongolism. *Acta Paediatr Scand* 68: 593-597, 1979. —Three girls with mosaic mongolism (46, XX/47, XX, +21) were followed cytogenetically and psychologically from the time of diagnosis at 8, 17 and 32 months of age, respectively, in the age of 13–14.5 years. All showed muscular hypotonia and hyperflexible joints at the time of diagnosis, but otherwise the physical characteristics of Down's syndrome were weakly expressed. The percentage of trisomic cells in the peripheral blood decreased with time, but were still higher in lymphocytes than in skin fibroblasts at the last investigation. Developmental milestones were delayed in all cases, and the developmental and intelligence quotients were decreased. Mental retardation was only slight in one of the cases (I.Q. = 65 at the age of 14 years 8 months). The intelligence quotients showed declining trends with time. Social ability and school results tended to be better than could be expected from the test results.

KEY WORDS Mosaic mongolism, mental development, chromosome analyses.

About 1–2% of all patients with Down's syndrome demonstrate a chromosomal mosaicism (9). Most cases have one cell line with an extra chromosome 21 and one with 46 normal chromosomes. Theoretically fewer aberrant cells should mean fewer and less pronounced symptoms of Down's syndrome. Early reports also pointed in that direction (2, 8) and were supported by the findings of mosaicism among some apparently healthy parents of children with trisomy 21 (10, 13). This opinion has later been questioned (7).

In order to get some further information about the mental development and frequency of trisomic cells at different ages in mosaic mongolism, three girls have been investigated on several occasions and the results are presented in this report.

CASE REPORTS

The clinical picture of the patients is presented in Fig. 1 and Table 1. Data on the mental development are given in Table 2.

Case 1 The proband was the second child of a 6-year-old mother and a 31-year-old father. Both parents and a brother had had a late psychomotor development. The mother walked at 24–26 months, the father at 17 months and the brother at 24 months of age. The brother's I.Q. (Terman-Merrill) was 65 at the age of 8 years.

The girl was born at term in 1958, weight 3770 g and length 50 cm. Abundant skin in the back of the neck was noted at birth. Down's syndrome was suspected a few months after birth and she was then admitted to a children's clinic for investigation. She had a flat and broad nasal bridge, slanting eyes, flat occiput, abundant skin in the back of the neck, generalized muscular hypotonia, hyperflexible joints, low normal Caffey's index (64) and retarded psychomotor development. Chromosome investigation was not done at this time and the definite diagnosis of mosaic mongolism was not made until the age of 6.5 years. A faint systolic murmur was heard at that time, but ECG and X-rays of the heart and lungs were normal. EEG showed scattered epileptogenic activity of sharp wave type. At the age of 15.5 years EEG showed diffuse abnormalities with slow basic rhythm without epileptogenic activity.

The girl's psychomotor development was late. She could sit at 11 months and walk at 24–25 months. Development and intelligence tests have shown a moderate mental retardation. I.Q. has tended to decrease with increasing age. The I.Q. (Terman-Merrill) was 51 and 41 at 6.4 and 15.5 years, respectively. Since the age of 7 years she has gone to a comprehensive school for mentally retarded

- stein J I MacDonald P C & Wilson J D Familial incomplete male pseudohermaphroditism type 2 Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadias *N Engl J Med* 291 944 1974
- 34 Wehmert J Zur Korrelation zwischen Hypospadie und weiteren angeborenen Missbildungen *Z Urol Nephrol* 67 887 1974
- 35 Wilson J D & Walther J D The conversion of testosterone to 5 α androst-17 β -ol 3-one (dihydro testosterone) by skin slices of man *J Clin Invest* 48 271 1969
- 36 Wray R C Ribando J M & Weeks P M The Byars hypospadias repair A review of 253 consecutive patients *Plast Reconstr Surg* 58 379 1976
- 37 Zurbrugg E F Inborn errors in testosterone biosynthesis with special reference to 17-oxo steroid reductase deficiency *Helv Paediatr Acta* Suppl 34 63 1974

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CASE REPORTS

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The girl was born at term in 1958, weight 3770 g and length 40 cm. Abundant skin in the back of the neck was noted at birth. Down's syndrome was suspected a few months after birth and she was then admitted to a children's clinic for investigation. She had a flat and broad nasal bridge, slanting eyes, flat occiput, abundant skin in the back of the neck, generalized muscular hypotonia, hyperflexible joints, low normal Caffey's index (64) and retarded psychomotor development. Chromosome investigation was not done at this time and the definite diagnosis of mosaic mongolism was not made until the age of 6.5 years. A faint systolic murmur was heard at that time, but ECG and X-rays of the heart and lungs were normal. EEG showed scattered epileptogenic activity of sharp wave type. At the age of 15.5 years EEG showed diffuse abnormalities with slow basic rhythm without epileptogenic activity.

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- 15 Wilson J D & Wilther J D. The conversion of testosterone to 5α androst-17 β ol-3-one (dihydrotestosterone) by skin slices of man. *J Clin Invest* 49: 271, 1969.
- 16 Wray R C, Ribando J M & Weeks P M. The Byars hypospadias repair. A review of 253 consecutive patients. *Plast Reconstr Surg* 59: 329, 1976.
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Table 2 *Mental development*

y=year(s) m=month(s) w=week(s)

	Age	Test	Result of test
Case 1	31 w	Gesell	Motonic
			Adaptive beha
			viour
			Language
			I Q =51
	6 y 5 m	Terman Merrill	I Q =43
			I Q =47
			I Q =41
Case	17 m	Gesell	Rough and fine
			motonic
			Adaptive beha
			viour
			Language
	7 m	Gesell	Rough motonic
			Fine motonic
			Adaptive beha
			viour
			Language
Case 3	14 y 8 m	Terman Merrill	I Q =65
	3 m	Gesell	Rough motonic
			Fine motonic
			Adaptive beha
			viour
			Language
	44 m	Terman Merrill	I Q =51
			I Q =38
	4 y 9 m	Terman Merrill	I Q =51
			I Q =38

the vitality of the child was good at birth. During the first days of life she was cyanotic peripherally the muscular tonus was decreased and the joints hyperflexible. Down's syndrome was not suspected.

She was referred to a children's clinic at the age of 7.5

years because of retarded psychomotor development and the diagnosis of mosaic mongolism was made. Physical findings of mongolism were slight slanting of the palpebral fissures, often protruding tongue, generalized muscular hypotonia and hyperflexible joints. ECG showed 1st

Table 3 *Chromosomal analyses of cells from peripheral blood (B) and skin (S)*

	Age	Tissue	Chromosome count				Total
			44	45	46	47	
Case 1	6.5 y	B	-	?	4	10 (19%)	54
		B	-	?	51	13 (0%)	66
		B	-	-	70	10 (13%)	80
		S	-	3	46	4 (7%)	53
		S	-	-	46	4 (8%)	50
Case	1.5 y	S	-	-	85	2 (11%)	87
		B	-	-	43	54 (56%)	97
		S	-	3	13	13 (43%)	31
		B	-	-	71	3 (13%)	24
		B	-	5	170	46 (7%)	171
Case 3	14.7 y	B	-	-	33	17 (34%)	50
		S	-	-	39	11 (7%)	50
		B	-	-	71	54 (43%)	125
		S	-	-	9	7 (19%)	36
		S	-	-	44	6 (17%)	50

47 XX +D

47 XX +G

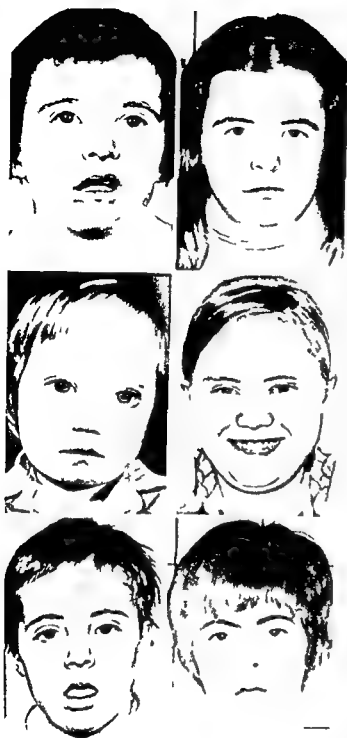


Fig. 1 Case 1, 2 and 3 at the ages of 6 y 6 m and 15 y 6 m, 2 y 3 m and 14 y 6 m, and 3 y 11 m and 13 y, respectively.

children, but transfer to a training school has been considered.

Case 2 Previously reported up to the age of 27 months (8). The proposita was the first child of a 38.5 year old mother and a 29 year old father. She was born after 40 weeks gestation, birthweight 2210 g and length 46 cm. She was observed at a children's hospital for a few days after birth because of moderate jaundice and because she

Table 1 Physical stigmata of Down's syndrome

++ Clearly present + slightly expressed - absent
● no information

	Case		
	1	2	3
Age at examination	6 y 6 m	2 y 3 m	3 y 11 m
Flattened occiput	++	●	●
Abundant neck skin	++	-	-
Oblique palpebral fissures	-	++	+
Epicanthal folds	-	+	-
Dysplastic ears	++	-	-
Flattened nasal bridge	+	++	-
Habitually open mouth	++	++	++
Large tongue	+	-	-
Protruding tongue	+	+	++
Irregular teeth	+	●	-
Short broad hands	+	-	-
Short curved 5th finger	-	-	-
Four finger crease	-	-	-
Increased spacing between 1st and 2nd toe	-	++	-
Muscular hypotonia	++	++	++
Hyperflexible joints	++	++	++

was lazy and did not suck satisfactorily. A slight generalized muscular hypotonia was noted but no obvious characteristics of mongolism.

At the age of 17 months she was investigated because of frequent upper respiratory tract infections and a suspicion of Down's syndrome. Physical examination showed a flat nasal bridge, slanting palpebral fissures, epicanthal fold on one side, often protruding tongue, generalized muscular hypotonia and hyperflexible joints. Caffey's index was low normal (65%). There were no signs of heart malformation. Chromosome analysis showed a mosaicism with normal and trisomy 21 cells. Clinical investigations at 27 months and 5.5 years, respectively, showed the same principal physical findings as at 17 months. EEG at 14.5 years was essentially normal.

The psychomotor development was late. She supported her head at 5-6 months and walked at 23 months. Developmental tests showed slight mental retardation, and at the age of 27 months the test results even pointed to a nearly normal intelligence. As in the other cases the result showed a declining trend with age. I.Q. (Terman Merrill) was 65 at 14.7 years of age. After one year in pre-school she started comprehensive school in a class for slow learners. At the age of 8 years. She has done well in school and has obtained average marks.

Case 3 The proposita was the first of two children to a 23.5 year old mother and a 31 year old father. The length of gestation was 40 weeks, birthweight 3110 g and length 52 cm. The amniotic fluid contained meconium but

Table 2 *Mental development*

y=year(s) m=month(s) w=week(s)

	Age	Test	Result of test	
Case 1	31 w	Gesell	Motonic	17-16 w
			Adaptive beha	16-20 w
			viour	24 w
			Language	
			I Q =51	
	6 y 5 m	Terman Merrill	I Q =43	
	7 y 11 m	Terman Merrill	I Q =47	
	11 y 6 m	Terman Merrill	I Q =41	
	15 y 6 m	Terman Merrill		
Case 2	17 m	Gesell	Rough and fine	9-10 m
			motonic	
			Adaptive beha	15-16 m
			viour	15 m
			Language	24 m
			Rough motonic	8 m
			Fine motonic	
			Adaptive beha	24-6 m
			viour	21 m
			Language	
			I Q =65	
Case 3	14 y 8 m	Terman Merrill		
	3 m	Gesell	Rough motonic	21-24 m
	44 m	Gesell	Fine motonic	21 m
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		S	-	-	39	11 (72%)	50
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	13 y	B	-	-	9	7 (19%)	36
	13 y	S	-	-	44	6 (17%)	50

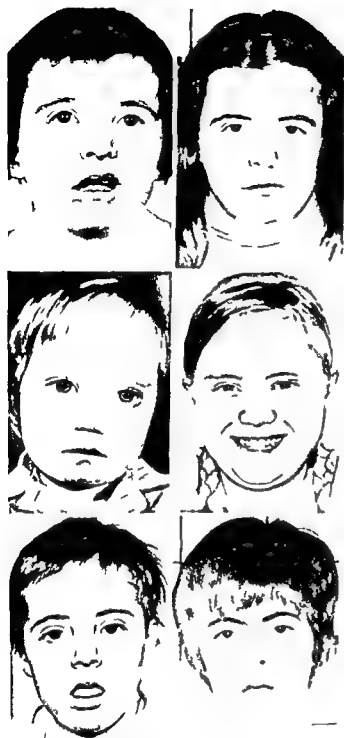


Fig. 1 Case 1 2 and 3 at the ages of 6 y 6 m and 15 y 6 m 2 y 3 m and 14 y 6 m and 3 y 11 m and 13 y respectively

children but transfer to a training school has been considered

Case 2 Previously reported up to the age of 27 months (8). The proposita was the first child of a 38.5 year old mother and a 29 year old father. She was born after 40 weeks gestation birthweight 2210 g and length 46 cm. She was observed at a children's hospital for a few days after birth because of moderate jaundice and because she

Table 1 Physical stigmata of Down's syndrome

++ Clearly present + slightly expressed - absent
● no information

	Case		
	1	2	3
Age at examination	6 y 6 m	2 y 3 m	3 y 11 m
Flattened occiput	++	●	●
Abundant neck skin	++	-	-
Oblique palpebral fissures	-	++	+
Epicanthal folds	-	+	-
Dysplastic ears	++	-	-
Flattened nasal bridge	+	++	+
Habitually open mouth	++	++	++
Large tongue	-	-	-
Protruding tongue	+	+	++
Irregular teeth	+	●	-
Short broad hands	+	-	-
Short curved 5th finger	-	-	-
Four finger crease	-	-	-
Increased spacing between 1st and 2nd toe	-	++	-
Muscular hypotonia	++	++	++
Hyperflexible joints	++	++	++

was lazy and did not suck satisfactorily. A slight generalized muscular hypotonia was noted but no obvious characteristics of mongolism.

At the age of 17 months she was investigated because of frequent upper respiratory tract infections and a suspicion of Down's syndrome. Physical examination showed flat nasal bridge, slanting palpebral fissures, epicanthal fold on one side, often protruding tongue, generalized muscular hypotonia and hyperflexible joints. Caffe index was low normal (65). There were no signs of heart malformation. Chromosome analysis showed a mosaicism with normal and trisomy 21 cells. Clinical investigation at 27 months and 5.5 years respectively showed the same principal physical findings as at 17 months. EEG at 14 years was essentially normal.

The psychomotor development was late. She supported her head at 5-6 months and walked at 23 months. Developmental tests showed slight mental retardation and at the age of 27 months the test results even pointed to nearly normal intelligence. As in the other cases the results showed a declining trend with age. IQ (Terman Merrill) was 65 at 14.7 years of age. After one year in pre school she started comprehensive school in a class for slow learners, i.e. at the age of 8 years. She has done well in school and has obtained average marks.

Case 3 The proposita was the first of two children to a 23.5 year old mother and a 31 year old father. The length of gestation was 40 weeks, birthweight 3110 g and length 52 cm. The amniotic fluid contained meconium but

ACKNOWLEDGMENT

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REFERENCES

- 1 Carr J Mental and motor development in young mongol children *J Ment Defic Res* 14 705 1970
- 2 Clarke C M Edwards J H & Smallpeice V 21 trisomy/normal mosaicism in an intelligent child with some mongoloid characters *Lancet* i 10 8 1961
- 3 Clarke C M Ford C E Edwards J H & Smallpeice V 21 trisomy/normal mosaicism in an intelligent child with some mongoloid characters *Lancet* ii 13 9 1963
- 4 Fishler K Koch R & Donnell G N Comparison of mental development in individuals with mosaic and trisomy 21 Down's syndrome *Pediatrics* 58 744 1976
- 5 Johnson R C & Abelson R B Intellectual behavior and physical characteristics associated with trisomy translocation and mosaic types of Down's syndrome *Am J Ment Defic* 73 85 1969
- 6 Koch R Share J Webb A & Graliker B V The predictability of Gesell developmental scales in mongolism *J Pediatr* 6 93 1963
- 7 Kohn G Taysi K Atkins T E & Mellman W J Mosaic mongolism I Clinical correlations *J Pediatr* 76 874 1970
- 8 Lindsten J Alvin A Gustavson K H & Fracaro M Chromosomal mosaic in a girl with some features of mongolism *Cytogenetics* 1 0 1967
- 9 Richards H W Mosaic mongolism *J Ment Defic Res* 13 66 1969
- 10 Smith H W Therman E M Putau K A & Inhorn E L Mosaicism in mother of two mongoloids *Am J Dis Child* 104 534 1967
- 11 Taylor A I Cell selection in vivo in normal/G trisomic mosaics *Nature* 219 10 8 1968
- 12 Taysi K Kohn G & Mellman W J Mosaic mongolism II Cytogenetic studies *J Pediatr* 76 880 1970
- 13 Weinstein E D & Warkany J Maternal mosaicism and Down's syndrome (mongolism) *J Pediatr* 63 599 1963
- 14 Zeeman D & House B J Mongoloid M A is proportional to log C A *Child Develop* 33 481 1962

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degree of AV block and EEG marked delta activity occipitally. At the age of 13 years EEG was apparently normal.

The psychomotor development was late. She sat at 10 months and walked at 24 months. Development and intelligence tests have shown a moderate mental retardation but with age the quotients showed a declining trend. At the ages of 2.7 and 3.7 years the development quotient (Gesell) was 70 and 58 respectively and at 4.7 and 12.9 years the IQ (Termin-Merrill) 51 and 38 respectively. After one and a half years in pre-school she started comprehensive school for mentally retarded children at the age of 8 years. She was transferred to a training school after two years because of difficulties in learning. The social adjustment has been very good.

CYTOGENETIC STUDIES

The results of the chromosome analysis were essentially the same in all cases. Two cell lines with 46 and 47 chromosomes respectively were found both in cultured lymphocytes and skin fibroblasts (Table 3). The cells with 46 chromosomes had a normal female karyotype while those with 47 chromosomes were trisomic for chromosome 21 (Q band analysis). The percentage of trisomy 21 cells was higher in the lymphocytes than in the skin fibroblasts in all investigations and decreased with increasing age of the patients both in the lymphocytes and the skin fibroblasts.

Chromosome analysis of the parents and sibs showed normal karyotypes.

DISCUSSION

Only a few reports of longitudinally followed cases with mosaic mongolism have been published (2, 3, 4, 7, 11, 12). None of these cases was followed for as long a time as those in the present study. The majority of the previously reported cases as well as the present three girls have shown a decrease in the frequency of trisomic cells in the peripheral blood and in skin fibroblasts. The frequency of trisomic cells was consistently higher in blood than in skin in all three patients. In contrast all cases of Taysi et al. (12) except one had a higher frequency of trisomic cells in skin fibroblasts. Selection for trisomic cells in long term skin cultures occurs (12) but should only

modify the figures. Probably the frequency of trisomic cells differs from tissue to tissue and the decreasing frequency reflects selection against the trisomic cell line.

The mental retardation in the present three girls progressed. Thus with increasing chronological age the development and intelligence quotient showed a declining trend. This is in accordance with what has been reported in non-mosaic (4, 6, 14) as well as in mosaic mongolism (3, 4, 7). Moreover the motorics in early age as in non-mosaic mongolism (1) tended to be more retarded than the mental development. Thus the mosaicism does not seem to change the general pattern of mental development. This is seen in non-mosaic mongolism.

There was a poor correlation between physical stigmata of Down's syndrome (5) frequency of trisomic cells (4, 12) and the degree of mental retardation in accordance with previous reports of mosaic mongolism. Case 1 had a low frequency of trisomic cells in the blood as well as in the skin, markedly lower than in the other cases. Despite of that the physical features of Down's syndrome were at least as much expressed as in the other patients and the IQ was low and declined with increasing chronological age. However additional factors might have played a role in this case since the girl's parents and brother also showed a late psychomotor development. In contrast case 2 had the highest frequency of trisomic cells in blood as well as in skin but she was only slightly mentally retarded.

Although the individuals with mosaic mongolism as a group have a better prognosis of mental development (4, 7) the overlapping in the distributions of the IQ in non-mosaic and mosaic mongolism is very high and as seen from cases 1 and 3 the prognosis is not necessarily better in mosaic than in non-mosaic mongolism. More exceptional cases such as case 2 who had an IQ of 65 at the age of 14.7 years are of course important to remember when talking to parents of a baby with mosaic

ADRENOCORTICAL FUNCTION IN PUBERTY

Serum ACTH Cortisol and Dehydroepiandrosterone in Girls and Boys

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ABSTRACT Apter D, Pakarinen A, Hammond G L and Viikko R (Department of Medical Chemistry, University of Helsinki, Helsinki and Department of Clinical Chemistry, University of Oulu, Oulu, Finland). Adrenocortical Function in Puberty. Serum ACTH, cortisol and dehydroepiandrosterone in girls and boys. *Acta Paediatr Scand* 1979; 68: 599-604. — Serum ACTH, cortisol and dehydroepiandrosterone (DHEA) were determined in 200 girls and 80 boys. In girls, serum DHEA showed significant increases between all bone age groups from the youngest one (7.5 years) to 15 years. A plateau was then seen up to 15.5 years of age, followed by a continuous increase to the oldest group (18.5 years). In boys, a progressive increase in DHEA was also seen from the youngest age group (8.5 years), but a period of a more rapid increase did not commence until after 12.5 years of age and it then continued to the oldest group. The level of DHEA in boys was significantly lower than in girls until the oldest group, in concert with the earlier pubertal development in girls. The importance of DHEA in initiating the early physical signs of normal puberty seems also to be different in the two sexes, since serum DHEA in girls was almost double that seen in boys when compared according to the stage of pubic hair growth. Serum cortisol showed a small progressive increase in girls, the concentrations postmenarche being significantly higher than premenarche. In boys, a decrease was seen up to 12.5 years of age and an increase occurred from 16.5 years onwards. In both sexes, ACTH and cortisol levels showed an inverse but non-significant relationship to each other. Serum ACTH levels in the different age groups showed no significant changes.

KEY WORDS Child development, menarche, radioimmunoassay, ACTH, cortisol, dehydroepiandrosterone.

The consensus of opinion seems to be that one of the earliest hormonal changes preceding the physical changes of puberty is activation of the adrenal cortex. This has been concluded from the increasing serum dehydroepiandrosterone and dehydroepiandrosterone sulphate concentrations observed as early as about 6 years of age in boys and girls (7, 8, 14-17). However, during puberty very little is known about the secretion of cortisol, the main corticoid secreted by the adrenal. The purpose of this study was to investigate adrenal function and its regulation in relation to pubertal changes. We report the concentrations of serum ACTH, cortisol and dehydroepiandrosterone in 200 girls and 80 boys. The boys

were examined on three occasions at one year intervals, and the girls twice at 1.5 year intervals.

MATERIALS AND METHODS

Subjects. Details of the female (3/4) and male (3/4) population have already been described, as well as the measurement of the physical parameters used (9, 18). In postmenarcheal girls, samples were obtained at days 6-9 of the cycle. All the samples were drawn at 08.00-10.00 a.m. In girls, bone age determination was carried out at each examination and therefore the data on girls are given in relation to bone age. In boys, bone age was not regularly recorded and the data are therefore given in relation to chronological age.

Hormone determinations. The estimation of serum ACTH was performed using CEA IRE SORIN (CIS) kits (Département des Rayonnements Ionisants, B.P. No. 71, 91190 Gif Sur Yvette, France). The methodo-

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MATERIALS AND METHODS

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Table 2 Serum DHEA, cortisol and ACTH in boys according to chronological age

	Age (years)										
	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.0-19.9
No. of subjects	7	14	18	17	18	3	16	19	18	17	14
DHEA $\mu\text{g/l}$											
Mean	0.55	1.09	1.0	1.70	1.66	2.44	3.7	3.14	3.46	4.69	6.41
S.E.M.	0.17	0.74	0.17	0.37	0.24	0.36	0.25	0.47	0.57	0.60	0.48
Cortisol $\mu\text{g/l}$											
Mean	87	99	10	86	64	64	60	66	67	101	10
S.E.M.	11	17	9	10	11	8	10	10	11	10	8
ACTH ng/l											
Mean	37	65	105	140	140	102	48	55	67	64	54
S.E.M.	11	70	27	40	48	31	8	17	27	19	8
Median	35	38	57	50	48	49	47	48	48	44	46
$p < 0.05$											
$p < 0.01$											
$p < 0.001$											

culated for each individual. The mean annual changes are calculated and then consecutively added to or subtracted from the value of the previous age group, beginning from the actual mean concentration of the age group of 13.5 years, which included the largest number of subjects. Table 1 gives the actual mean concentrations of all the samples analyzed in annual bone age groups for the three hormones in a cross sectional manner.

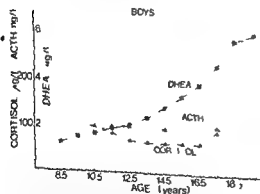


Fig. 2 Serum DHEA, cortisol and ACTH in pubertal boys. The changes have been expressed in a mixed longitudinal way (see ref. 10). In this case the means of the individual annual changes are successively added to or subtracted from the mean actual concentration of the age group of 13.5 years. This point is the one representing the actual mean of the age group; others reflect relative changes.

DHEA shows an increase in the youngest age groups of girls (Fig. 1, Table 1). Its concentration is significantly increased up to 12.5 years of age, after which a plateau is seen which extends until 15.5 years of age. The plateau is again followed by a continuous increase.

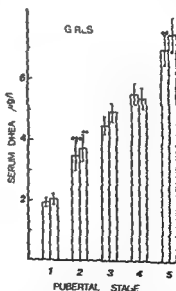


Fig. 3 Serum DHEA according to breast (light columns) and pubic hair (dark columns) stages in girls. One to three of the symbols \circ or \times indicate $p < 0.05$, $p < 0.01$, $p < 0.001$ significances of difference in respect to the preceding stage of breast and pubic hair development respectively. The bars indicate ± 1 standard error of the mean. The number of subjects in each group is 79-54 girls.

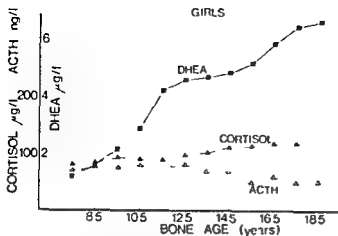


Fig. 1 Serum DHEA, cortisol and ACTH in pubertal girls. The changes have been expressed in a mixed longitudinal manner (see ref. 10) in which the ratios of changes in hormone concentrations and bone age in each individual are first calculated. The mean annual changes are then added to or subtracted from the value of the previous age group beginning from the mean actual concentration of the age group of 13.5 years, which included the largest number of subjects. This point is the one representing the actual mean of the age group; others reflect relative changes.

logies outlined in the manufacturer's instructions were followed and 0.1–0.2 ml samples of serum were used. Individual assays were monitored by the inclusion of low, medium and high controls in each batch of unknown samples. Each batch also included samples from all age groups, and all samples from the same person were determined in the same series. In our hands, the lowest detectable amount which could be measured reproducibly was 7.2 ng/l when 0.1 ml of serum was used. The intra-assay coefficients of variation were 15% (21 ng/l).

11% (72 ng/l) and 7% (310 ng/l). The interassay coefficient of variation was 17% (68 ng/l, $n=11$).

Serum cortisol was determined by a highly specific method as previously described (2). In short, the method is as follows: Serum (0.1 ml) is diluted with water, extracted with diethyl ether/ethyl acetate (90:10 by vol) and chromatographed on a microcolumn of Lipdex 5000™ (hydroxyalkoxypropyl Sephadex). Part of the cortisol fraction was taken for radioimmunoassay using a highly specific antibody prepared against cortisol-³H hemisuccinate bovine serum albumin (BSA) as the antigen.

Dehydroepiandrosterone was determined in duplicate 50 µl serum samples after extraction with 1 ml of petroleum ether and using an automated approach similar to that for some sex steroids as outlined previously (11).

The antibody was raised against 3 β -hydroxy-7 α -carboxymethyl- Δ^4 -androst-17-one BSA. Characteristics of the antibody and details of the radioimmunoassay of dehydroepiandrosterone are described in Hammond et al. (12).

The statistical treatment of the results was performed at the Department of Data Processing, University of Oulu. Groups were compared using the two-tailed unpaired Student's *t* test or the Wilcoxon test for paired differences.

RESULTS

Fig. 1 shows the changes in serum ACTH, cortisol and dehydroepiandrosterone (DHEA) in girls expressed by a mixed longitudinal approach (modified from Gupta, 10). In this approach, the ratios of individual changes in hormone concentration and bone age are calculated.

Table 1 Serum DHEA, cortisol and ACTH in girls according to bone age

In postmenarcheal girls, the values are from samples taken between days 6–9 of the menstrual cycle

	Bone age (years)											
	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5
No. of subjects	7	16	12	27	18	24	33	18	28	33	25	7
DHEA µg/l												
Mean	0.87	1.34	1.55	2.68	3.49	4.60	4.78	5.10	4.97	6.94	7.30	6.93
S.E.M.	0.11	0.14	0.12	0.20	0.16	0.18	0.19	0.15	0.15	0.66	0.83	0.59
Cortisol µg/l												
Mean	82	80	83	99	87	100	103	121	116	132	130	129
S.E.M.	15	7	8	8	10	7	7	10	7	6	8	14
ACTH ng/l												
Mean	85	68	59	64	118	82	69	88	81	63	60	50
S.E.M.	16	11	9	6	33	13	10	14	10	6	5	14
Median	69	64	52	64	59	63	55	67	62	50	54	36

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

of normal puberty seems also to be different in the two sexes. In the case of pubic hair (Figs 3 and 4) the level of serum DHEA in girls is almost double (160–180%) that seen in boys at each stage. Quantitatively greater increases in testosterone or a higher sensitivity of the target tissues for androgenic stimulus in boys may be responsible for this sex difference.

The changes in serum cortisol are not very marked. In girls a progressive but slow increase is observed throughout the age groups studied. The changes seen in boys although small follow a somewhat different pattern. Serum cortisol first displays a significant decrease until about 12 years of age and an increase takes place after 16.5 years of age. The serum cortisol concentrations found by us are close to those recently reported by Ducharme et al (8). Their results also show similar patterns of pubertal changes in serum cortisol. Quite recently Parker et al have presented data showing a tendency to increasing serum cortisol concentrations in the course of puberty but the changes were not statistically significant (15). Kenny et al (13) found an increase in cortisol production rate in the course of puberty but only in proportion to the increase in body size.

Only small and statistically insignificant changes are seen in serum ACTH. The changes seem to be inversely related to those of serum cortisol.

On the basis of the data obtained early activation of the adrenal cortex preceding puberty involves only a restricted part of its activities and so far has been reported to be reflected only in the secretion of DHEA (and its sulphate) (7, 8, 14–17) and pregnenolone (4). It is important to emphasize that there is a sex difference in this function as found in this and an earlier study (8). Serum FSH and prolactin also display changes which are different in the two sexes and herald the onset of puberty (3). The interaction of prolactin and the adrenal cortex in puberty is possible because elevated prolactin production leads to

elevated serum DHEA which returns to normal along with the normalization of serum prolactin (5, 6, 19). However the small physiological changes of serum prolactin in infants and during the years prior to puberty especially in boys (1) make it unlikely that prolactin alone is of importance in the initiation of puberty.

REFERENCES

- 1 Aubert M L, Sizonenko M C, Kaplan S L & Grumbach M M. The ontogenesis of human prolactin from fetal life to puberty. In P G Crosignani & C Robyn (eds) *Prolactin and human reproduction*. Academic Press, London, New York, San Francisco, 1977, p 9.
- 2 Apter D, Janne O & Vihko R. Lipidex chromatography in the radioimmunoassay of serum and urinary cortisol. *Clin Chim Acta* 63: 139, 1975.
- 3 Apter D, Pakarinen A & Vihko R. Serum prolactin, FSH and LH during puberty in girls and boys. *Acta Paediatr Scand* 67: 417, 1978.
- 4 Apter D & Vihko R. Serum pregnenolone, progesterone, 17 hydroxyprogesterone, testosterone and 5 α -dihydrotestosterone during female puberty. *J Clin Endocrinol Metab* 45: 1039, 1977.
- 5 Bassi F, Giusti G, Borsì L, Cattaneo S, Gianotti P, Forti G, Pazzagli M, Vigiani C & Serio M. Plasma androgens in women with hyperprolactinemic amenorrhoea. *Clin Endocrinol* 6: 5, 1977.
- 6 Carter J N, Tyson J E, Warne G L, McNeill A S, Faiman C & Friesen H G. Adrenocortical function in hyperprolactinemic women. *J Clin Endocrinol Metab* 45: 973, 1977.
- 7 de Peretti E & Forest M G. Unconjugated dehydroepiandrosterone plasma levels in normal subjects from birth to adolescence in human. The use of a sensitive radioimmunoassay. *J Clin Endocrinol Metab* 43: 98, 1976.
- 8 Ducharme J R, Forest M G, de Peretti E, Sempe M, Collu R & Bertrand J. Plasma adrenal and gonadal sex steroids in human pubertal development. *J Clin Endocrinol Metab* 47: 468, 1976.
- 9 Greulich W W & Pyle S J. *Radiographic atlas of skeletal development of the hand and wrist*. Stanford University Press, Stanford, 1959.
- 10 Gupta D A. Longitudinal study of steroid excretion patterns in children during adolescent growth. *Steroidologia* 1: 767, 1970.
- 11 Hammond G L, Vannikka L & Vihko R. Automation of radioimmunoassays for some sex steroids with use of both iodinated and tritiated ligands. *Clin Chem* 23: 1750, 1977.
- 12 Hammond G L, Kovisto M, Kouvalainen K & Vihko R. Serum steroids and pituitary hormones in neonatal infants with particular reference to the decline in testicular activity in infant boys. Submitted for publication, 1978.

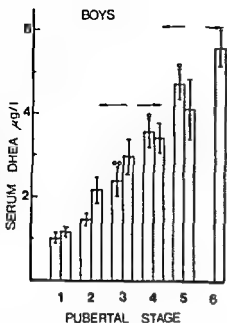


Fig. 4 Serum DHEA according to external genitalia (light columns) and pubic hair (dark columns) stages in boys. One to three of the symbols O or X indicate $p < 0.05$, $p < 0.01$, $p < 0.001$ significance of differences in respect to the preceding stage or the stages marked by an arrow. The bars indicate ± 1 standard error of the mean. The number of subjects in each group is 15–75 boys.

crease until 18.5 years, which was the oldest age group studied.

Considerably less change is seen in the serum levels of ACTH and cortisol. None of the changes seen in serum ACTH (Fig. 1) are significant but there seems to be a slight decrease in serum ACTH concentration after 12.5 years of age. Simultaneously, serum cortisol increases (Table 1, Fig. 1) suggesting a change in the regulation of the interaction of these two hormones. When the girls are divided into premenarcheal and postmenarcheal groups, serum cortisol is 92 ± 35 (S.D.) $\mu\text{g/l}$ ($n=115$) and 125 ± 37 (S.D.) $\mu\text{g/l}$ ($n=130$) respectively. The latter value is significantly higher ($p < 0.001$).

The corresponding data for the boys are shown in Fig. 2 and Table 2. Serum DHEA also increases in the youngest boys, but a period of a more rapid increase does not commence until after 12.5 years of age. The increase continues without the appearance of a plateau over the age groups investigated.

Serum cortisol decreases significantly ($p <$

0.01) from 10.5 years of age until about 12.5 years of age. After 16.5 years of age there is a similar relative change in serum ACTH and cortisol in boys as there is in girls (Figs. 1 and 2). ACTH decreases while cortisol increases.

A continuous increase is seen in serum DHEA in girls according to pubic hair and breast stages (Fig. 3). The same is true in boys when they are grouped according to external genitalia and pubic hair stages (Fig. 4). At the same pubic hair stages the girls have significantly higher concentrations of DHEA than boys ($p < 0.01$) at each stage (see Figs. 3 and 4). The girls also show higher levels in each annual age group ($p < 0.05$ or < 0.01) with the exception of the oldest age group (Tables 1 and 2).

In both girls and boys serum cortisol correlates with DHEA ($p < 0.001$) but not with ACTH.

DISCUSSION

To obtain more information on adrenal function and its regulation in the course of puberty we investigated serum DHEA, ACTH and cortisol in boys and girls 7–19 years of age.

The effect of diurnal variation was minimized by always drawing blood samples at 08.00–10.00 h. To avoid stress effect on adrenal function no physical training was allowed on the morning of the investigation. Also the nature of the study was carefully explained to the participants in advance to avoid psychological stress. The large number of subjects investigated also diminishes the effect of stress on the final results.

In accordance with earlier studies (7, 8, 14–17) we found that an increase in serum DHEA takes place very early. In girls a significant increase was seen between the 7.5 and 8.5 year old groups according to bone age and an earlier increase cannot be ruled out. Although slightly increasing values are also seen in the youngest boys, the period of a more rapid increase appears later than in girls. The importance of DHEA in the early physical signs

PEAK EXPIRATORY FLOW RATE

Reference Values for Swedish Children

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ABSTRACT Bjure J, Dalén G and Kjellman B (Department of Paediatric Clinical Physiology, East Hospital, Gothenburg and Department of Paediatrics, Karnsjukhuset, Skövde, Sweden.) Peak expiratory flow rate. Reference values for Swedish children. *Acta Paediatr Scand* 68:605-1979.—Reference values for the peak expiratory flow rate assessed by the Wright-McKerrow peak flow meter have been established for Swedish children. The material consisted of 143 boys and 132 girls. We recommend the sexes be considered together. The equation of the regression line is $72.14 \times \text{height}^2 + 96$. The coefficient of correlation is 0.93 and the residual standard deviation 13.7 $\text{L} \cdot \text{min}^{-1}$.

KEY WORDS Healthy children, peak expiratory flow rate

The peak expiratory flow meter (PEFM) described by Wright & McKerrow (7) is frequently used when examining children with pulmonary diseases. Reference values for the peak expiratory flow rate (PEFR) assessed by the PEFM have been established for children by various authors (2-4, 5). One of these studies covers Danish children but none Swedish children (2).

Table 1 presents the ages and heights of the children. The principles of selection were the same for both groups and the same as in a previous study (1). All of the children were investigated in the sitting position. One and the same PEFM (for adults) was used for the children of group A and another PEFM (for adults) in group B. Five or more good forced expiratory maneuvers were done by the children in group A and three in group B. The highest values received in each case were used for the calculations.

MATERIALS AND METHODS

Group A: 117 boys and 110 girls selected from an ordinary school and investigated at the Department of Paediatric Clinical Physiology, East Hospital, Gothenburg.

Group B: 31 boys and 111 girls selected from an ordinary school and investigated at the Department of Paediatrics, Karnsjukhuset, Skövde.

RESULTS AND COMMENTS

Group A had a small but significantly higher mean age ($p < 0.05$) than group B whereas there were no significant differences concerning height and weight.

The equations of the regression lines of

Table 1 Two groups of healthy children investigated with the Wright peak flow meter

Group	n	Age (years)		Height (cm)	
		Mean	Range	Mean	Range
A boys	11	11.5	7-17.0		
A girl	10	11.7	7.0-17.9	149	117-186
B boys	31	10.9	7.5-14.8	148	114-174
B girl	30	10.7	7.3-15.0	147	111-180
				144	119-17

- 13 Kenny F M Adrenal function in adolescence In F P Heald & W Hung (eds) *Adolescent endocrinology* Appleton Century Crofts New York 1970 p 127
- 14 Korth Schutz E Levine L S & New M J Serum androgens in normal prepubertal and pubertal children and in children with precocious adrenarche *J Clin Endocrinol Metab* 47 117 1976
- 15 Parker L N Sack J Fisher D A & Odell W D The adrenarche Prolactin gonadotropins adrenal androgens and cortisol *J Clin Endocrinol Metab* 46 396 1978
- 16 Sizonenko P C & Paunier L Hormonal changes in puberty III Correlation of plasma dehydroepiandrosterone testosterone FSH and LH with stages of puberty and bone age in normal boys and girls and in patients with Addison's disease or hypogonadism or with premature or late adrenarche *J Clin Endocrinol Metab* 41 894 1975
- 17 Sizonenko P C Paunier L & Cirmignac D Hormonal changes during puberty IV Longitudinal study of adrenal androgen secretions *Hormone Res* 7 269 1976
- 18 Tanner J M Growth and endocrinology of the adolescent In L J Gardner (ed) *Endocrine and genetic diseases of childhood and adolescence* W B Saunders Company Philadelphia London Toronto 1975 2nd ed p 14
- 19 Vermeulen A Suy F & Rubens R Effect of prolactin on plasma DHEA(S) levels *J Clin Endocrinol Metab* 44 1222 1977

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REFERENCES

- 1 Dolen G & Kjellman B: Evaluation of three spirometers on healthy children *Acta Paediatr Scand* In press 1978
- 2 Juhl H: Pulmonary function investigations on 1011 school children using Wright's peak flow meter *Scand J Clin Lab Invest* 25: 355 1970
- 3 Mantel N J *Am Stat Assoc Inst* 27: 719 1963
- 4 Murray A B & Cook C H: Measurement of peak expiratory flow in 20 normal children from 4.5 to 18.5 years of age *J Pediatr* 67: 186 1963
- 5 Nairn I R, Bennet A I, Andrew I H & MacArthur J: A study of respiratory function in normal school children. The peak flow rate *Arch Dis Child* 36: 53 1961
- 6 Oden A & Wedel H: Arguments for Fishers permutation test *Ann Stat* 3: 518 1975
- 7 Wright B H & McKerrow C H: Maximum forced expiratory flow as a measure of ventilatory capacity. With description of a new portable instrument for measuring it *Br Med J* 11: 1041 1959

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Table 2 Equations of the regression lines of peak expiratory flow rate (PEFR l/min) to height in metre raised to the third power (H^3) and coefficients of correlation (r) for two groups of healthy children

Residual standard deviations around the lines (R S D) are also given as l/min and as percentages of the mean values

Group	Equation of the line PEFR =	r	R S D (l/min)	R S D (%)
A total	$71.95 \times H^3 + 101.99$	0.93	46.0	13.2
A boys	$68.89 \times H^3 + 110.79$	0.94	45.7	13.1
A girls	$77.11 \times H^3 + 86.51$	0.93	46.3	13.4
B total	$67.70 \times H^3 + 91.86$	0.91	42.7	14.0
B boys	$69.80 \times H^3 + 90.39$	0.92	46.5	14.6
B girls	$61.42 \times H^3 + 105.47$	0.84	38.9	13.3
A+B total	$72.14 \times H^3 + 96.12$	0.93	46.2	13.7

PEFR with respect to height (raised to the third power) are given in Table 2. To test if the PEFR values of group A differed significantly from the values of group B for a given height and sex a nonparametric partial analysis was performed. The groups were divided in a number of intervals with respect to sex and height. Within each such interval Fisher's permutation test variable was determined (6). The test variables from the different intervals of height were pooled (3). The values of the total group A (boys and girls) and that of the girls of group A were found to be significantly higher ($p < 0.01$) than the corresponding values of group B. No significant difference was found for the boys of the two groups.

PEFR values for three heights calculated from the equations are given in Table 3. Cor-

responding values given by three other studies are also shown for comparison.

The correlation coefficients and residual standard deviations (R S D) in our study are similar to those found in other studies (2, 4, 5).

The variations between different studies have several possible causes e.g. differences of race, of selection and composition of the groups, of the procedure used and the cooperation of the subject and differences between the various PEFM used. As the differences between groups A and B are small compared to the great R S D and the uncertainty factors we consider it suitable to combine our two groups and recommend the sexes be considered together as Naam et al (5). As reference data for Swedish children we recommend total groups A and B (Table 3).

Table 3 Predicted mean peak expiratory flow rate (PEFR) of healthy children for the heights 130 cm, 145 cm and 160 cm

The results of four studies are given. R S D = residual standard deviations around the lines as percentages of the mean values

Investigation	n	R S D (%)		PEFR at three heights					
				130 cm		145 cm		160 cm	
		♂	♀	♂	♀	♂	♀	♂	♀
Present Group A	214	13.1	13.4	262	256	321	322	393	407
Present group B	61	14.6	13.3	244	240	303	293	376	357
Present groups A + B	275	13.7		255		316		392	
Juhl (1970)	779	12.9	12.2	242	237	321	311	399	385
Murray & Cook (1963)	220	14.5	14.3	261	261	347	330	437	400
Naam et al (1961)	471	13.4		251	282	335	362	418	447

HEMOGLOBIN A₁ (HbA_{1c}) IN CHILDREN WITH LONG STANDING AND NEWLY DIAGNOSED DIABETES MELLITUS

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ABSTRACT Heinze E Kohne E Meissner C Beischer W Teller W M and Klehauer E (Department of Pediatrics University of Ulm/Donau F R G) Hemoglobin A₁ (HbA₁) in children with long standing and newly diagnosed diabetes mellitus Acta Paediatr Scand 68 609 1979—In 35 children with long standing diabetes mellitus a significant correlation was found between the hemoglobin A₁ (HbA₁) and the 24 hour urinary glucose excretion By contrast 11 newly diagnosed diabetic children had grossly elevated HbA₁ concentrations but no correlations could be established between the levels of HbA₁ and the duration of symptoms blood glucose glycosuria ketonuria and the acid-base status However HbA₁ and C peptide were significantly correlated The elevated HbA₁ concentrations decreased towards normal in all of these 11 children after 2-3 months following adequate therapy The results suggest that the determination of HbA₁ may serve as a valuable metabolic control index in children with long standing diabetes mellitus but adds little information in newly diagnosed diabetic patients For the individual diabetic child during the early treatment period HbA₁ may be the index of choice for adequacy of metabolic control

KEY WORDS Diabetes mellitus children HbA₁ metabolic control

Rabhar Blumenfeld & Ramney (16) were the first to report an elevation of hemoglobin A₁ (HbA₁) in diabetic patients This was confirmed by others and in addition a close correlation between the metabolic control of the disease and the elevation of HbA₁ seemed established (4 8 12 13 14)

In the present study HbA₁ was determined in two divided groups of diabetic children in one group were children with diabetes of more than one year's duration in the other group HbA₁ was measured sequentially from the time of diagnosis at various time intervals following the onset of treatment

MATERIAL AND METHODS

Patients Forty six diabetic children aged 15 to 18 years were studied According to the duration of their disease they were divided into two groups (repetition see above) as with diabetes over one

year's duration (range 1-15 years) Group two consisted of 11 diabetic children who were regularly studied from the time of diagnosis and throughout the following months of therapy Pertinent clinical and laboratory data are presented in Table I

All 46 children received a diet which contained 40-45% of calories as carbohydrate 35-40% fat and 15-20% protein insulin replacement was achieved with a mixture of a regular and an intermediate insulin preparation twice a day The amount of insulin was adjusted according to the urinary glucose excretion which was tested at home three times a day by Clinitest[®] All patients were regularly followed in the outpatient clinic A 24-hour urine was collected at home utilizing random samplings i.e. in the morning the afternoon and the night

Methods Blood glucose was measured with a hexokinase method (16) the capillary pH and the P_{CO₂} by routine analysis the urine sugar polarimetrically and the acetone excretion with Acetest[®] The immunoreactive C peptide was determined as previously described (1) HbA₁ was measured by column chromatography according to Huisman & Meyering (10) with minor modifications (11)

The correlation coefficient was calculated with a Hewlett Packard calculator 9810A connected with a Hewlett Packard calculator plotter 9860A to draw the regression line automatically

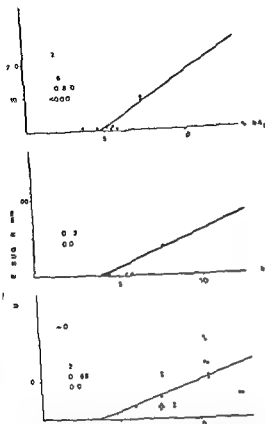


Fig 7 Correlations between HbA_{1c} and the urinary glucose in diabetic children with long standing diabetes mellitus at two months (—) one month (—) prior and at the time (0) of the Hb determination

(3-8) glucagon free insulin growth hormone (8) and the thickness of the basement membrane (12). There was some degree of correlation in some cases between HbA_{1c} concentrations and cholesterol as well as triglyceride but none was found between the former and hormones or changes of the basement membrane.

No significant correlations were forthcoming between the HbA_{1c} levels and the duration of symptoms, the blood and urine glucose concentrations, the acid-base status or the ketone body excretion in the present study of the 11 newly diagnosed diabetic children. However, the positive correlation between the C peptide and the HbA_{1c} concentrations was unexpected.

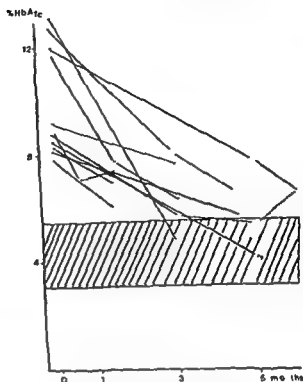


Fig 8 Pattern of the HbA_{1c} concentration in 11 diabetic children from the time of diagnosis and during the following months. The shaded area indicated the normal range.

Very recently highly specific insulin receptors have been identified on human erythrocytes. There are as yet few but controversial findings relating to the physiological role of insulin on the glucose transport system of human red cells (7). On the other hand, since insulin is released in equimolar amounts with C peptide from the B cells, it may well be that insulin modulates the intracellular glucose concentration in the human red cells. No correlation was found between the blood glucose and the C peptide concentration which underscores the insensitivity of the B cells in juvenile diabetics to glucose (9).

It should be emphasized that all determinations were performed in the non fasting state which could explain the discrepancies between our data and those of a recent study in newly diagnosed juvenile diabetics (3). These serum indices were determined in the

Table 1 Clinical and laboratory data of 11 newly diagnosed diabetic children on admission

Case	Sex	Age (years)	Onset-weeks	Blood glucose (mg/dl)	pH	Base exc (mval/l)	Urine sugar (mmol/l)	Acetest*	C Pep (ng/ml)	HbA _{1c} (%)
1	M	10.5	6	223	7.26	-9	361	++	0.7	9.7
2	F	12.5	2	225	-	-	600	+++	0.8	8.8
3	M	14.5	1	1167	6.95	-24	416	+++	<0.5	8.9
4	F	11.5	5	240	-	-	222	+	0.8	7.8
5	F	1.5	6	240	7.38	-4	194	++	<0.5	8.3
6	M	11	4	370	7.04	-24	278	+++	0.9	12.0
7	F	13.5	20	223	7.46	+2	378	+	0.0	11.8
8	F	3.2	3	235	-	-	355	++	0.8	13.1
9	M	10.5	0.5	460	7.39	-4	539	+++	0.7	8.4
10	M	9.2	3	225	-	-	417	++	<0.5	6.9
11	F	11.5	8	430	7.35	-6	522	++	0.2	12.7
<i>r</i>			0.392	0.204	0.155	0.712	0.036	0.022	0.656	
<i>P</i>			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05	

* = correlation coefficients versus HbA_{1c}

RESULTS

Fig. 1 shows the results of the patients from group one of more than one year's duration. The urine sugar concentrations (mmol/l) represent the mean of the three separately collected specimens. Hemoglobin A_{1c} (HbA_{1c}) was measured and the regression analyses were plotted against the urine glucose concentrations simultaneously with (0 months) and one month (-1 month) and two months (-2 months) prior to the determination of the glycosylated hemoglobin A. A significant correlation was found between the urine glucose concentrations and HbA_{1c}.

In group two the correlation coefficients were calculated between HbA_{1c} concentrations and the following parameters: the time when the first symptoms suggestive of diabetes mellitus were noted by the patients; the initial blood glucose concentrations; the pH; the base excess; the urine glucose; the acetone excretion; and the immunoreactive serum C peptide concentrations (Table 1). A correlation of statistical significance was only found between the HbA_{1c} and the C peptide concentrations, while the correlation coefficient between the C peptide and the blood glucose levels was $r=0.148$.

During the following months, when the 11 diabetic children were treated effectively with

diet and insulin, the grossly elevated HbA_{1c} concentrations declined in all patients and reached the normal range in 3 children (Fig. 2).

DISCUSSION

During recent years, numerous authors could show that the elevated HbA_{1c} concentrations correlated very well with the blood and the urinary glucose concentrations in juvenile and adult onset diabetes (4, 8, 12, 13, 14).

In the present study, the results obtained in the 35 children who suffered from diabetes mellitus for more than one year confirm the previous reports. Furthermore, Gabbay et al. (6) found the highest correlation between the glycosylated hemoglobin and the amount of glucose excretion in the specimens collected 2 months prior to the determination of HbA_{1c}. It was suggested that the overwhelming formation of HbA_{1c} predominantly occurs during the first half of the 120-day life span of the red cells. The observation that a plateau of the HbA_{1c} concentration was reached in 60- to 80-day old erythrocytes tends to support this conclusion (2).

To further characterize the metabolic profile in the diabetic patients, correlations were looked for between the glycosylated hemoglobin and cholesterol (3, 6, 8), triglycerides

LETTER TO THE EDITOR

PLASMA PREALBUMIN IN THE NEWBORN

Sir

We read with interest the paper of Jacobsen and associates (1). The authors emphasize that in new born infants with birth weights appropriate for gestation serum prealbumin concentrations increase progressively with gestational age and that in small for gestational age babies the levels are significantly lower. Recently we reported on the same subject and could also find that in pre term infants plasma prealbumin at birth increases with foetal age (3). However only one out of nine small for dates new born infants had a plasma prealbumin level below the normal range. Also our recent data shown in Table 1 and presented according to the findings of Jacobsen et al

(1) do not suggest that plasma prealbumin is a reliable index of foetal malnutrition. As plasma levels in control male adults 29.7 (1) versus 27.7 (3) mg/dl are similar in both studies the apparent discrepancy can hardly be explained by technical differences. From the data presented in both studies it seems also unlikely that the observed discrepancy is due to differences in sampling time: first postnatal week (1) and at birth only (3). However it is noteworthy that only our study compares appropriate for dates and small for dates pre term infants and that the statistical analysis of our data shows a high variance in the group of small for dates full term infants which suggests high individual differences in the study group.

Table 1 Plasma prealbumin (mg/dl) in the newborn

	Pre term infants (≤ 36 weeks)		Full term infants (37-41 weeks)	
	AGA	SGA	AGA	SGF
Number	64	10	76	8
Mean	8.10	7	11.5	10.5
SEM	0.43	0.93	0.69	1.93
Variance	17	8.7	17.40	79.70

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REFERENCES

1. Jacobsen H, Pettersen B, Andersen H, J & Hummer L. Serum concentrations of thyroxine binding globulin, prealbumin and albumin in healthy fullterm, small for gestational age and preterm newborn infants. *Acta Paediatr Scand* 68:49 1979.
2. Lubchenco L O. *The high risk infant*. Saunders Company Philadelphia 1976 1st ed p 65.
3. Socha J, Eggermont E, Carchon H, Devlieger H & Eeckels W. Plasma prealbumin in low birthweight infants. *Acta Paediatr Belg* 30:171 1977.

Appropriate for gestational age newborns (AGA) have birth weights between P_{10} and P_{90} for foetal age, small for gestational age infants (SGA) have birth weights below P_{10} for foetal age (2). There is no statistical difference between AGA and SGA pre term infants or between AGA and SGA full term infants. Statistical differences ($p < 0.001$) are found only between AGA pre term and AGA full term newborns and between SGA pre term and AGA full term babies.

fasting state and significant correlations were found between glucose cholesterol tri glyceride on the one hand and the HbA_{1c} concentration on the other

Our results do suggest however that determinations of HbA_{1c} give no further information about the acute metabolic situation in newly diagnosed juvenile onset diabetes. Furthermore assuming that the glycosylation of hemoglobin A in diabetes occurs under similar conditions as in normals with a plateau of HbA_{1c} concentration in 60-80-day old red cells it appears that a considerable degree of hyperglycemia may be tolerated without any clinical symptoms suggestive of diabetes mellitus (2-5)

During remission induced by appropriate treatment the elevated HbA_{1c} concentrations declined in all 11 recent onset diabetes. This suggests that the glycosylated hemoglobin is an excellent indicator for the metabolic control in the individual patient during the remission phase of juvenile onset diabetes mellitus.

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REFERENCES

- Beischer W, Keller L, Mias M, Schiefer E, Pfeiffer E F. Human C-peptide. Part I. Radioimmuno assay. *Klin Wochenschr* 54: 709 1976
- Bunn H F, Haney D N, Kamin S, Gabbay K H, Gallop P M. The biosynthesis of human hemoglobin A₁. Slow glycosylation of hemoglobin in vivo. *J Clin Invest* 57: 1652 1976
- Ditzel J & Kjaergaard J J. Hemoglobin A₁ concentration for newly discovered diabetes. *Br Med J* 1: 741 1978
- Dolhofer R, Stadel A & Wieland O H. Clinical and biochemical studies on the significance and formation of hemoglobin A₁ and A_{1c} in diabetes mellitus. *Klin Wochenschr* 55: 945 1977
- Fitzgibbons J F, Koler R D & Jones R T. Red cell age related changes in hemoglobins A₁ and A_{1c} in normal and diabetic subjects. *J Clin Invest* 58: 820 1976
- Gabbay K H, Hasty K, Breslow J L, Ellison R C, Bunn H F & Gallop P M. Glycosylated hemoglobins and long term blood glucose control in diabetes mellitus. *J Clin Endocrinol Metab* 44: 859 1977
- Gambhir K K, Archer J A & Bradley D J. Characteristics of human erythrocyte insulin receptors. *Diabetes* 27: 701 1978
- Gonen B, Rochman H, Rubenstein A H, Tanega S P & Horwitz D L. Haemoglobin A₁. An indicator of the metabolic control of diabetic patients. *Lancet* II: 734 1977
- Heinze E, Beischer W, Keller L, Winkler G, Teller W M & Pfeiffer E F. C-peptide secretion during the remission phase of juvenile diabetes. *Diabetes* 27: 670 1978
- Huisman T H J & Meyering C H. Studies on the heterogeneity of hemoglobin I. The heterogeneity of different human hemoglobin types in carboxymethylcellulose and in Amberlite IRC 50 chromatography. Quantitative aspects. *Clin Chim Acta* 5: 103 1960
- Huisman T H & Janvis J H P. The hemoglobinopathies. Techniques of identification. *Clinical and biochemical analysis*. Marcel Dekker Inc. New York and Basel 1977 vol 6 pp 126-131
- Koenig R J, Peterson C M, Kilo C, Cerami A & Williamson J R. Hemoglobin A_{1c} as an indicator of the degree of glucose intolerance in diabetes. *Diabetes* 25: 230 1976
- Lanoe R, Thibault N, Eschwege E, Soria J, Soria C & Tchobronsky G. Glycosylated haemoglobin concentrations and clinical results in insulin-dependent diabetes. *Lancet* II: 1156 1977
- Paulsen E P. Hemoglobin A₁ in childhood diabetes. *Metabolism* 22: 269 1973
- Rahbar S, Blumenfeld O & Ranney H M. Studies of an unusual hemoglobin in patients with diabetes mellitus. *Biochem Biophys Res Commun* 36: 838 1969
- Schmidt F H. Enzymatische Methode zur Bestimmung von Blut und Harnzucker unter Berücksichtigung von Vergleichsuntersuchungen mit klassischen Methoden. *Internist* 4: 554 1963

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LETTER TO THE EDITOR

Sir

Although the mechanism proposed by Dr Katznelson (1) for the increased intracranial pressure observed in three infants with cystic fibrosis cannot be discounted another explanation is possible. It has been reported previously (2) that a deficiency of vitamin A may predispose to development of increased intracranial pressure and bulging fontanelles in cystic fibrosis. In fact we have observed a similar syndrome in infants who have been malnourished prior to the diagnosis of cystic fibrosis and who do not have severe respiratory distress. Dr Katznelson does not mention whether his three patients were treated with enzymes and/or vitamins during their hospital course. If so this could account perhaps for the decrease in intracranial pressure noted.

Finally it is somewhat difficult to understand how such marked airway obstruction which produced increased intracranial pressure could occur with preservation of arterial blood gases. The relatively high P_{O_2} and appropriately low P_{aCO_2} in cases 1 and 2 leads one to believe that the airway obstruction could not have been excessive at least in these two patients.

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REFERENCES

1. Katznelson D. Increased intracranial pressure in cystic fibrosis. *Acta Paediatr Scand* 67: 607, 1978.
2. Keating J H & Feigin R D. Increased intracranial pressure associated with probable vitamin A deficiency in cystic fibrosis. *Pediatrics* 46: 41, 1970.

The Editor has asked Dr Katznelson to comment on this letter

I want to thank Dr Taussig for drawing attention to deficiency of vitamin A as yet another cause for increased intracranial pressure. Vitamin A and carotene blood levels were not determined in our cases and therefore obviously this possibility cannot be excluded at least in two of the cases. Case 2 had a bulging fontanel at the age of one month. This is possibly too short a period in which to develop significant avitaminosis in our infant who had been perfectly normal nutritionally at birth. In the paper referred to by Dr Taussig (1) both infants had had overt clinical signs of vitamin A deficiency. None of our cases had had xerophthalmia.

Dr Taussig's suggestion that the improvement in the intracranial pressure in our cases was really the result of the unknowing associated administration of vitamin A and not to the improvement in the pulmonary status as I proposed fails to note the clinical observation that there had been a rise and fall in the bulge of the fontanel concomitantly with the increase and decrease of respiratory obstruction signs. This cycle occurred twice in Case 2 and three times in Case 3 and all in the space of a few days. Had the first episode of bulging fontanel been due to avitaminosis A and its alleviation the result of therapy then yet another mechanism must be marshalled to explain the subsequent episodes of recurring bulge of the fontanel.

As to the question of the severity of airway obstruction the clinical reversible picture of severe tachypnea with poor air entry, clear lung fields on X ray with overdistended lungs and small heart appear most suggestive of air

SHORT COMMUNICATION

PLASMA LEVELS OF COMPLEMENT FACTORS 3 AND 4 OROSOMUCOID AND OPSONIC FUNCTIONS IN ANOREXIA NERVOSA

Previous studies have shown that various alterations of the defence against infectious agents can occur in anorexia nervosa patients (6-10). In a case report Kim & Michael (5) described changes in the serum complement system including depression of C3 while C4 remained normal. Since nothing is known about the frequency of alterations in the complement system and other acute phase reactants in anorexia nervosa, 10 consecutive patients have been studied with regard to the plasma levels of complement factors 3 and 4 (C3 & C4), orosomucoid and the opsonic functions of plasma for PMN granulocytes.

The anorexia nervosa patients (9 girls and one boy (for details see refs 3-10)) conformed to previously described criteria for the disease (3). The mean age was 14.7 years and the mean weight loss was 27% from pre-morbid weight. All patients but one were below -2 S.D. relating weight to height. Fifteen normal weight controls were investigated simultaneously. Details concerning them have been published previously (10).

Heparinized plasma samples from the patients and controls were frozen at -50°C within 2 hours of withdrawal and thawed only just prior to assay. C3, C4 and orosomucoid levels were determined by an electro-immunoassay (7 of 11).

The opsonic function of plasma was analysed with an assay for PMN bactericidal activity (9). PMNs from healthy donors were incubated with *Staph. aureus* and 1% plasma for 90 min. The results are expressed as the percentages living CFU of the initial CFU counts.

The anorexia patients had a significantly lower mean value ($p < 0.001$) for their plasma C3 levels than the controls (Fig. 1). Five of

the 10 patients showed values outside the reference values (mean ± 2 S.D.). No significant differences between patients and controls were found for C4 and orosomucoid levels and opsonic functions of plasma (Fig. 1). Nor were any significant correlations found in the anorexia patients between, on the one hand, values for the C3, C4 and orosomucoid levels and on the other, for weight, weight loss from pre-morbid weight and opsonic functions (Student's t test and linear regression).

The present C3 decreases could be due to a diminished synthesis, changes in the distribu-

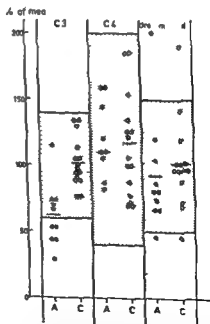


Fig. 1 Plasma C3, C4 and orosomucoid concentrations in anorexia nervosa patients (A, ●) and in normal weight controls (C, ○). Mean value —. The shaded area represents the reference values (mean ± 2 S.D.). The opsonic function test (mean values \pm S.E.): anorexia patients $8.9 \pm 0.6\%$ living CFU; controls $7.4 \pm 0.6\%$ (n.s.).

way obstruction in C F. In Case 3 the blood gas levels are in keeping with this. In Case 1 the P_{aCO_2} was only mildly low (P_{aCO_2} 4.7 kPa). It is only in Case 2 that the P_{aCO_2} was very low (P_{aCO_2} 3.1 kPa). This of course must have been the result of the hyperventilation itself indicative of the degree of respiratory embarrassment and refers to the time of ad-

mission and not to the situation three days later when the fontanel bulged. It is not impossible that the P_{aCO_2} may have been higher.

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SHORT COMMUNICATION

PLASMA LEVELS OF COMPLEMENT FACTORS 3 AND 4 OROSOMUCOID AND OPSONIC FUNCTIONS IN ANOREXIA NERVOSA

Previous studies have shown that various alterations of the defence against infectious agents can occur in anorexia nervosa patients (6-10). In a case report Kim & Michael (5) described changes in the serum complement system including depression of C3 while C4 remained normal. Since nothing is known about the frequency of alterations in the complement system and other acute phase reactants in anorexia nervosa, 10 consecutive patients have been studied with regard to the plasma levels of complement factors 3 and 4 (C3 & C4), orosomucoid and the opsonic functions of plasma for PMN granulocytes.

The anorexia nervosa patients (9 girls and one boy) (for details see refs 3-10) conformed to previously described criteria for the disease (3). The mean age was 14.7 years and the mean weight loss was 27% from pre-morbid weight. All patients but one were below -2 S.D. relating weight to height. Fifteen normal weight controls were investigated simultaneously. Details concerning them have been published previously (10).

Heparinized plasma samples from the patients and controls were frozen at -50°C within 2 hours of withdrawal and thawed only just prior to assay. C3, C4 and orosomucoid levels were determined by an electro-immunoassay (7, cf 11).

The opsonic function of plasma was analysed with an assay for PMN bactericidal activity (9). PMNs from healthy donors were incubated with *Staph aureus* and 1% plasma for 90 min. The results are expressed as the percentages living CFU of the initial CFU counts.

The anorexia patients had a significantly lower mean value ($p < 0.001$) for their plasma C3 levels than the controls (Fig. 1). Five of

the 10 patients showed values outside the reference values (mean ± 2 S.D.). No significant differences between patients and controls were found for C4 and orosomucoid levels and opsonic functions of plasma (Fig. 1). Nor were any significant correlations found in the anorexia patients between on the one hand values for the C3, C4 and orosomucoid levels and on the other for weight, weight loss from pre-morbid weight and opsonic functions (Student's t test and linear regression).

The present C3 decreases could be due to a diminished synthesis, changes in the distribu-

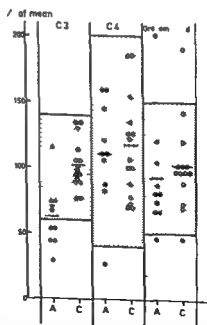


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tion between different compartments or in increased catabolism. The data in the present study do not indicate which mechanism(s) might be responsible. Although decreased synthesis has usually been suggested as the mechanism responsible for low C3 levels in studies on undernourished patients from developing countries, these have also shown signs of an increased C3 consumption and increased levels of other acute phase reactants, probably secondary to coexisting infections (2, 8, 13). In this study, however, normal C4 and orosomucoid levels together with absence of clinical signs of infections or other diseases argue from the anorexia nervosa rather than from the classical or alternative pathway, less likely. Hence, a diminished synthesis is a probable explanation for the low C3 levels (cf. 5).

Decreased serum levels of acute phase reactants have been reported in undernourished subjects and in fasting (8, 9, 11). The anorectic patients showed normal orosomucoid levels, however, which could be due to the absence of coexisting infections and the fact that protein depletion is of minor importance in this disorder compared with other forms of undernutrition (1).

The finding of a normal opsonic function of 1% plasma despite low C3 levels also agrees with previous studies on chronically undernourished subjects and findings in total fasting (9, 12, cf. however 4) where opsonic functions have most often been normal.

It is concluded that in anorexia nervosa low C3 levels together with decreased granulocyte functions (10) might contribute to a decreased resistance to e.g. bacterial infections.

REFERENCES

1. Aperia A, Broberger O & Fohlin L. Renal function in anorexia nervosa. *Acta Paediatr Scand* 67: 219, 1978.
2. Chandra R K. Serum complement components in malnourished Indian children. In R M Suskind (ed.) *Malnutrition and the Immune Response*. Raven Press, New York, 1977, p. 379.
3. Fohlin L. Body composition, cardiovascular and renal function in adolescent patients with anorexia nervosa. *Acta Paediatr Scand* 66 (Suppl. 768): 1977.
4. Keusch G T, Urrutia J J, Fernandez R, Guerrero O & Casteneda G. Humoral and cellular aspects of intracellular bacterial killing in Guatemalan children with protein-calorie malnutrition. In R M Suskind (ed.) *Malnutrition and the Immune Response*. Raven Press, New York, 1977, p. 745.
5. Kim Y & Michael A F. Hypocomplementemia in anorexia nervosa. *J Pediatr* 87: 587, 1975.
6. Hjosen B, Basso H H & Myking O. The glucose oxidation in isolated leukocytes from female patients suffering from overweight or anorexia nervosa. *Scand J Clin Lab Invest* 35: 447, 1975.
7. Laurell C B. Electroimmunoassay. *Scand J Clin Lab Invest* 29 (Suppl. 174): 21, 1972.
8. McFarlane H. Acute phase proteins in malnutrition. In R M Suskind (ed.) *Malnutrition and the Immune Response*. Raven Press, New York, 1977, p. 401.
9. Palmblad J. Fasting (acute energy deprivation) in man: effect on polymorphonuclear granulocyte functions, plasma iron and serum transferrin. *Scand J Hematol* 17: 217, 1976.
10. Palmblad J, Fohlin L & Lundström M. Anorexia nervosa and polymorphonuclear (PMN) granulocyte reactions. *Scand J Hematol* 19: 334, 1977.
11. Palmblad J, Cantell K, Holm G, Norberg R, Sirén H & Sundblad L. Acute energy deprivation in man: effect on serum immunoglobulins, antibody response, complement factors 3 & 4, acute phase reactants and interferon-producing capacity of blood lymphocytes. *Clin Exp Immunol* 30: 40, 1977.
12. Seth V & Chandra R K. Opsonic activity, phagocytosis and bactericidal capacity of polymorphs in undernutrition. *Arch Dis Child* 47: 282, 1977.
13. Sirisinha R, Suskind R M, Edelman R, Kulapongs P & Olson R E. The complement system in protein-calorie malnutrition—a review. In R M Suskind (ed.) *Malnutrition and the Immune Response*. Raven Press, New York, 1977, p. 309.

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SHORT COMMUNICATION

HEREDITARY TYROSINAEMIA AND DIABETES MELLITUS

The main features in hereditary tyrosinaemia are liver damage leading to cirrhosis and renal tubular defects with hypophosphataemic rickets (4). Pain attacks in the abdomen and legs similar to those seen in acute intermittent porphyria and hypertensive crisis have been reported in this disorder (4). We report here a boy with similar symptoms and signs but who also had diabetes mellitus.

Patient

Boy, first child to healthy unrelated parents, no heredity for diabetes mellitus. Birth weight 3180 g. After weaning at 3 months he vomited and had a peculiar odour. Admitted at 6 months because of fever and abdominal pain. He had hepatosplenomegaly and foetor hepaticus. Reduced liver produced coagulation factors (e.g. P & P 5th) hypophosphataemia (0.8 mmol/l), glucosuria, generalized aminoaciduria with high excretion of methionine, tyrosine and phenolic acids, increased urinary excretion of 8-aminolevulinic acid, porphobilinogen in urine, negative P-tyrosine and P-methionine increased (Table 1). Phenylalanine tolerance test (100 mg/kg body weight) gave a maximum value of 11 tyrosine of 883 μ mol/l at 5 h and a slow decrease to 557 μ mol/l at 7 h. Correspondingly an increased urinary excretion of phenolic acids was observed. Fructose tolerance test (50 mg/kg) was normal. P-ceruloplasmin and P-orosomucoid levels were remarkably low and P-albumin moderately decreased (Table 1). Skeletal X-ray showed no signs of rickets. He was treated with a tyrosine and phenylalanine restricted diet supplemented with iron, ascorbic acid, vitamin B₁₂ and other vitamins. Marked clinical improvement with increasing levels of coagulation factors (e.g. P & P 7th) normophosphataemia and reduced levels of P-tyrosine but P-ceruloplasmin and P-orosomucoid were still low (Table 1). From 15 months hypophosphataemia increasing urinary volume no glucosuria. At 19 months increasing thirst and large urine volume hyperglycaemia (45 mmol/l), glucosuria but no ketoacidosis. P-insulin 3 μ E/ml. The same low level of P-ceruloplasmin and P-orosomucoid otherwise normal plasma protein electrophoresis (Table 1). Insulin-dependent diabetes mellitus was diagnosed requiring 17 U insulin per day. The diet was modified to satisfy as far as possible the demands of the two diseases. During the next year he was in a remarkably good condition and had a normal motoric and mental development. His weight was 15 kg and height 111 cm at age 5 years. At 7½ years he had his first hypokalaemic crisis (2.9 mmol/l) with

acidosis. At 3 years pain in abdomen and legs, ketoacidosis and hypokalaemia, blood pressure 130/100 mmHg. Urinary volume 3-4 l/day. Insulin requirement between 10 and 20 U per day. P-ceruloplasmin and P-orosomucoid decreased (Table 1). During the next three years he had 17 similar attacks with pains predominantly in the legs, hypokalaemia (lowest value 1.4 mmol/l), hypophosphataemia, renal acidosis and urinary volume up to 7 l/day. At four years radiological signs of rickets and also some pathological fractures. Increasing muscle wastage and inability to walk. Supplementary large doses of potassium, vitamin B₁₂, phosphate and bicarbonate were tried orally. At 8 years he also had a hypertensive crisis, blood pressure 160/170 mmHg and cerebral symptoms. Increased levels of P-aldosterone, P-renin and increased urinary excretion of aldosterone but not of catecholamines. At 6½ years he had a hypertensive crisis 230/140 mmHg with encephalopathy and increased urinary excretion of aldosterone and catecholamines. He died in a picture of cardiac failure. Autopsy was made 48 h after death. Advanced autolytic changes made a detailed microscopical investigation impossible. The main gross lesions were a nodular cirrhosis of the liver, a hypertrophy of the left side of the heart and slight signs of cardiac failure with a moderate pulmonary edema and marked congestion of the liver and the spleen. As far as could be seen the islets of Langerhans and the suprarenal glands were normal. Some crystalline granules were found in the renal tubular epithelium (Autopsy performed by Dr I. Gliberg, Department of Pathology, Malmö General Hospital, Malmö).

This patient fulfils the diagnostic criteria for hereditary tyrosinaemia of the acute type (7). He also had hypertensive crisis, intermittent pains and increased levels of P-aldosterone and P-renin. The mechanism for these abnormalities will be discussed more fully elsewhere (to be published).

The isolated decrease of P-ceruloplasmin and P-orosomucoid has not been reported earlier in this disorder. The cause of this abnormality is obscure.

Diabetes mellitus has not been reported before in hereditary tyrosinaemia. Several of the biochemical abnormalities in hereditary tyrosinaemia, such as hypokalaemia, acidosis, hypophosphataemia and hypermethioninaemia

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REFERENCES

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- 3 Fohlin L. Body composition, cardiovascular and renal function in adolescent patients with anorexia nervosa. *Acta Paediatr Scand* 66 Suppl. 1977.
- 4 Keusch G T, Urrutia J J, Fernandez R, Guerrero O & Casteneda G. Humoral and cellular aspects of intracellular bacterial killing in Guatemalan children with protein-calorie malnutrition. In R M Suskind (ed.) *Malnutrition and the Immune Response*. Raven Press, New York, 1977, p. 145.
- 5 Kim Y & Michael A F. Hypocomplementemia in anorexia nervosa. *J Pediatr* 87: 982, 1975.
- 6 Kjosen B, Bassoe H H & Myking O. The glucose oxidation in isolated leukocytes from female patients suffering from overweight or anorexia nervosa. *Scand J Clin Lab Invest* 35: 447, 1975.
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- 9 Palmblad J. Fasting (acute energy deprivation) in man: effect on polymorphonuclear granulocyte functions, plasma iron and serum transferrin. *Scand J Hematol* 17: 217, 1976.
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- 11 Palmblad J, Cantell K, Holm G, Norberg R, Strander H & Sundblad L. Acute energy deprivation in man: effect on serum immunoglobulins, antibody response, complement factors 3 & 4, acute phase reactants and interferon producing capacity of blood lymphocytes. *Clin Exp Immunol* 30: 50, 1977.
- 12 Seth V & Chandra R K. Opsonic activity, phagocytosis and bactericidal capacity of polymorphs in undernutrition. *Arch Dis Child* 47: 282, 1972.
- 13 Sinsinhal S, Suskind R M, Edelman R, Kulapongs P & Olson R E. The complement system in protein-calorie malnutrition—a review. In R M Suskind (ed.) *Malnutrition and the Immune Response*. Raven Press, New York, 1977, p. 309.

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SHORT COMMUNICATION

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	Reference values (range)		Patient				
	9 m-2 y	4 y-6 y	6 m	1 y	1 y 7 m	3 y	6 y
Tyrosine	11-122	25-59	311	166	65	76	76
Phenylalanine	23-69	18-67	86	76	63	66	46
Methionine	3-29	11-26	496			34	
Isoleucine	26-94	40-61	19		22	85	
Leucine	45-155	75-115	33		31	151	
Arginine	11-65	40-102	45		117	171	
Ceruloplasmin	0.22-0.48		0.06	0.06	0.06	0.15	
Orosomucoid	0.55-1.05		0.16	0.16	0.13	0.37	
Albumin	40-52		31	40	39	43	

may also theoretically contribute to the pathogenesis of diabetes mellitus.

A hereditary tyrosinaemia like syndrome i.e. elevated blood tyrosine and methionine levels and pancreatic islet hyperplasia and degeneration was produced by feeding guinea pigs methionine (6). In Chinese hamsters imbalance in the content of sulphhydryl (SH) containing amino acids caused by excess amounts of methionine (administered intraperitoneally) induced decreased granulation and necrosis of the pancreatic β cells (1) and hyperglycaemia and glucosuria (2). Hypermethioninaemia is also found in patients with homocystinuria. Hyperinsulinism and also pre-diabetic oral and intravenous glucose tolerance tests have been observed in such patients (2, 3). Furthermore it is well known that several amino acids (mainly arginine) stimulate the secretion of insulin. In hereditary tyrosinaemia the pancreatic islets are hyperplastic and hypoglycaemia has been reported (4). Thus it is possible that in hereditary tyrosinaemia after an initial stimulation of the β -cells an exhaustion occurs with hypoinsulinism and ensuing diabetes mellitus.

The boy had also hypophosphataemia. This leads to a state of endogenous insulin resistance (5). This mechanism might have con-

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REFERENCES

- Boquist L. The effect of excess methionine on the pancreas. A light and electron microscopic study in the Chinese hamster with particular reference to degenerative changes. *Lab Invest* 21: 96, 1969.
- Falkmer S, Boquist L, Eriksson L, Helgesson K, Holmgren G & Noren E. Is hypermethioninaemia diabetogenic? A clinical study in patients with homocystinuria supplemented by experimental investigations in the Chinese hamster. *Acta Endocrinol* 87 Suppl 203: 18, 1976.
- Holmgren G, Falkmer S & Hambræus L. Plasma insulin content and glucose tolerance in homocystinuria. *Upsala J Med Sci* 78: 215, 1973.
- La Du NN & Gessing LR. Tyrosinosis and tyrosinaemia. In JB Stanbury, JB Wyngaarden & DS Fredrickson (eds.) *The metabolic basis of inherited disease* 4th ed. McGraw Hill Book Co New York 1978 p. 256.
- Marshall WP, Banasiak MF & Kalkhoff R. Effects of phosphate deprivation on carbohydrate metabolism. *Horm Metab Res* 10: 369, 1978.
- Perry TL, Hardwick DF, Hansen S, Pohlmann L & Warrington PD. Methionine induction of experimental tyrosinaemia. *J Mental Def Res* 11: 246, 1967.

CASE REPORT

ELECTROLYTE ABNORMALITIES IN LYMPHOSARCOMA
AFTER CHEMOTHERAPYF TZORTZATOU C DACOU VOUTETAKIS S HAIDAS
F PAPADELLIS and TH THOMAIDIS*From the first Department of Paediatrics of Athens University
St Sophie's Children's Hospital Athens Greece*

ABSTRACT Tzortzidou F, Dacou Voutetakis C, Haidas S, Papadellis F and Thomaidis Th (First Department of Paediatrics of Athens University, St Sophie's Children's Hospital Athens Greece). Electrolyte abnormalities in lymphosarcoma after chemotherapy. *Acta Paediatr Scand* 68: 621-623 1979.—A 12 year-old female with lymphosarcoma responding to treatment including vincristine and cyclophosphamide developed clinical and laboratory findings compatible with the syndrome of inappropriate secretion of antidiuretic hormone. Some additional findings were observed i.e. uremia, hypotension and alkalosis that have not so far been recorded in that syndrome. All abnormalities were corrected upon water restriction. A similar episode occurred after a 2nd drug course. It too was corrected upon water restriction. The patient was clinically free from her malignancy in both episodes. It is suggested that our child had probably an expanded form of the syndrome of inappropriate secretion of antidiuretic hormone.

KEY WORDS Inappropriate secretion of antidiuretic hormone, vincristine, cyclophosphamide.

Hyponatremia and water intoxication have been described in patients with various malignancies and have been attributed to oversecretion of antidiuretic hormone (ADH) or ADH-like substances by tumour cells (4). Vincristine and/or cyclophosphamide toxicity have also been incriminated as causes of the inappropriate antidiuretic hormone secretion syndrome (SIADH) (3, 7, 8).

The present report concerns a 12 year-old female with a lymphosarcoma in therapeutic remission who presented the syndrome of inappropriate secretion of antidiuretic hormone. Attention is drawn to a few laboratory features not so far recorded.

CASE REPORT

A 12 year-old female presented with lymphosarcoma of the left parotid gland, proven by biopsy which regressed completely after radiotherapy. The parents

refused chemotherapy at that time. Three months later the patient was first admitted to our service because of a mass arising from the liver, pallor, anorexia and weight loss. Her admission body weight was 79 kg. Investigations showed the following: Hemoglobin 70 g/l, leucocytes 8.6 $\times 10^9$ /l, neutrophils 94%, lymphocytes 5%, eosinophils 1%. Platelet count was normal as were bone marrow smear, chest X-ray and intravenous pyelography. Urine analysis revealed no pathologic changes, specific gravity was 1.010. Total serum bilirubin was 35.9 μ mol/l, unconjugated bilirubin was 22.2 μ mol/l, SGPT was 34 units and SGOT 100 units. Blood urea nitrogen was 6.4 mmol/l. She was considered to have a metastasis in the liver. She was given a blood transfusion, prednisone 4 mg per kg of body weight for 5 days and the following chemotherapy in one i.v. dose: cyclophosphamide 600 mg per m² of body surface i.e. 750 mg, vincristine 1.7 mg per m² i.e. 14 mg and adriamycin 40 mg per m² i.e. 50 mg. Two weeks later chemotherapy was repeated once more. The liver mass had disappeared completely.

Twelve days after the 2nd dose of chemotherapy the patient was anorectic and presented an acute episode of vomiting, dyspnea and cyanosis. The blood pressure was 60/40 mmHg, the pulse was weak and the pulse rate was 80 per min. She was started on i.v. fluid. Electrocardiogram showed S-T changes suggestive of myocardial ischaemia and chest X-ray was normal. Two hours later

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REFERENCES

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- Holmgren G, Falkmer S & Hambræus L. Plasma insulin content and glucose tolerance in homocystinuria. *Uppsala J Med Sci* 78: 215 1973.
- La Du BN & Gessing L. Tyrosinosis and tyrosinaemia. In J B Stanbury, J B Wyngaarden & D S Fredrickson (eds.) *The metabolic basis of inherited disease*. 4th ed. McGraw Hill Book Co. New York 1978 p 256.
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F TZORTZATOU C DACOU VOUTETAKIS S HAIDAS
F PAPADELLIS and TH THOMAIDIS*From the first Department of Pediatrics of Athens University
St Sophie's Children's Hospital Athens Greece*

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KEY WORDS Inappropriate secretion of antidiuretic hormone, vincristine, cyclophosphamide.

Hyponatremia and water intoxication have been described in patients with various malignancies and have been attributed to oversecretion of antidiuretic hormone (ADH) or ADH like substances by tumour cells (4). Vincristine and/or cyclophosphamide toxicity have also been incriminated as causes of the inappropriate antidiuretic hormone secretion syndrome (SIADH) (3, 7, 8).

The present report concerns a 12 year-old female with a lymphosarcoma in therapeutic remission who presented the syndrome of inappropriate secretion of antidiuretic hormone. Attention is drawn to a few laboratory features not so far recorded.

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A 12 year-old female presented with lymphosarcoma of the left parotid gland proven by biopsy which regressed completely after radiotherapy. The parents

refused chemotherapy at that time. Three months later the patient was first admitted to our service because of a mass arising from the liver, pallor, anorexia and weight loss. Her admission body weight was 79 kg. Investigations showed the following: Hemoglobin 70 g/l, leucocytes $8.6 \times 10^9/l$, neutrophils 94%, lymphocytes 5%, eosinophils 1%. Platelet count was normal as were bone marrow smear, chest X-ray and intravenous pyelography. Urine analysis revealed no pathologic changes. Specific gravity was 1.010. Total serum bilirubin was $35.9 \mu\text{mol/l}$, unconjugated bilirubin was $27.2 \mu\text{mol/l}$, SGPT was 34 units and SGOT 100 units. Blood urea nitrogen was 6.4 mmol/l. She was considered to have a metastasis in the liver. She was given a blood transfusion, prednisone 2 mg per kg of body weight for 5 days and the following chemotherapy in one i.v. dose: cyclophosphamide 600 mg per m^2 of body surface, i.e. 750 mg, vincristine 1.7 mg per m^2 , i.e. 14 mg and adriamycin 40 mg per m^2 , i.e. 50 mg. Two weeks later chemotherapy was repeated once more. The liver mass had disappeared completely.

Twelve days after the 2nd dose of chemotherapy the patient was anorectic and presented an acute episode of vomiting, dyspnea and cyanosis. The blood pressure was 60/40 mmHg, the pulse was weak and the pulse rate was 80 per min. She was started on i.v. fluids. Electrocardiogram showed S-T changes suggestive of myocardial ischaemia and chest X-ray was normal. Two hours later

Table 1 Amino acid concentration in plasma ($\mu\text{mol/l}$) and ceruloplasmin, orosomucoid and albumin in plasma (g/l) at various ages

	Reference values (range)		Patient				
	9 m-2 y	4 y-6 y	6 m	1 y	1 y 7 m	3 y	6 y
Tyrosine	11-122	25-58	311	166	65	2/6	761
Phenylalanine	23-69	38-67	86	36	63	66	46
Methionine	3-29	11-26	496			34	
Isoleucine	76-94	40-63	19		22	85	
Leucine	45-155	75-115	33		31	151	
Arginine	11-65	40-102	45		117	121	
Ceruloplasmin	0.23-0.48		0.06	0.06	0.06	0.15	
Orosomucoid	0.55-1.05		0.16	0.16	0.13	0.33	
Albumin	40-52		31	40	39	43	

min can theoretically contribute to the pathogenesis of diabetes mellitus.

A hereditary tyrosinemia-like syndrome i.e. elevated blood tyrosine and methionine levels and pancreatic islet hyperplasia and degeneration was produced by feeding guinea pigs methionine (6). In Chinese hamsters imbalanced in the content of sulphhydryl (SH) containing amino acids caused by excess amounts of methionine (administered intraperitoneally) induced decreased granulation and necrosis of the pancreatic β cells (1) and hyperglycemia and glucosuria (2). Hypermethioninemia is also found in patients with homocystinuria. Hyperinsulinism and also pre-diabetic oral and intravenous glucose tolerance tests have been observed in such patients (2, 3). Furthermore it is well known that several amino acids (mainly arginine) stimulate the secretion of insulin. In hereditary tyrosinemia the pancreatic islets are hyperplastic and hypoglycemia has been reported (4). Thus it is possible that in hereditary tyrosinemia after an initial stimulation of the β cells an exhaustion occurs with hypoinsulinism and ensuing diabetes mellitus.

The boy had also hypophosphatemia. This leads to a state of endogenous insulin resistance (5). This mechanism might have con-

tributed to the exhaustion of the pancreatic β -cells.

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REFERENCES

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- Falkmer S, Boquist L, Eriksson L, Helgeson K, Holmgren G & Norén E. Is hypermethioninaemia diabetogenic? A clinical study in patients with homocystinuria supplemented by experimental investigations in the Chinese hamster. *Acta Endocrinol* 97 Suppl 703 1B 1976.
- Holmgren G, Falkmer S & Hambræus L. Plasma insulin content and glucose tolerance in homocystinuria. *Upsala J Med Sci* 78: 215 1973.
- La Du B N & Gyessing L E. Tyrosinosis and tyrosinaemia. In J B Stanbury, J B Wyngaarden & D S Fredrickson (eds.) *The metabolic basis of inherited disease* 4th ed. McGraw Hill Book Co New York 1978 p 256.
- Marshall W P, Banasak M F & Kalkhoff R K. Effects of phosphate deprivation on carbohydrate metabolism. *Horm Metab Res* 10: 369 1978.
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DISCUSSION

The accepted clinical and laboratory criteria for the diagnosis of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are the following: (a) hyponatraemia and hypo-osmolality of serum and extracellular fluid; (b) increased urinary sodium and hyper osmolality in relation to that of serum; (c) low serum chloride; (d) no clinical evidence of hypovolaemia, dehydration and edema; (e) no renal nor adrenal disease and (f) improvement of the above upon water restriction (4, 6, 7).

Our child probably represents a case of SIADH since she satisfies all of the above criteria. Her severely depleted electrolytes were indeed not restored by intravenous administration of 0.9% NaCl solution and KCl they were corrected by simple restriction of fluids. On the other hand the absence of albuminuria, glucosuria, hyposthenuria and increased serum creatinine exclude renal dis-

ease, hypopotassemia without polyuria would also tend to exclude adrenal disease.

Our child however had in addition uremia, low serum potassium (2.5 mmol/l) and metabolic alkalosis (CO_2 62.4 mmol/l and blood pH 7.55). Whether these represent an expansion of the syndrome itself or some other associated abnormalities of obscure nature cannot be stated at present. Uremia was not renal since it was transient and not accompanied with other renal findings. It was observed only during both episodes. Low serum potassium has been observed in leukemia and other early or advanced lymphocellular malignancies (4) as well as after prolonged antibiotic administration (5). Our child did not receive antibiotics and her malignancy was in definite remission at the time. Alkalosis has been said to occur secondary to low serum potassium as a compensatory mechanism or as others believe hypopotassemia is due to the alkalosis itself. What is however impressive is the disappearance of uremia, hypopotassemia and alkalosis upon water restriction and the subsequent remission of the syndrome. The clinical and laboratory features of this syndrome not only disappeared after water restriction but reappeared when the same chemotherapy was given again a few weeks later to be restored once more after water restriction. During the 2nd episode uremia, hypopotassemia and alkalosis were also present. ECG changes were attributed to adriamycin and not to hypopotassemia since the latter was corrected whereas the ECG changes persisted. As far as causation is concerned a multitude of factors have been incriminated. SIADH may be due to the tumour itself (4) or to some other pathological state. It is believed that tumour cells synthesize and secrete a substance that simulates antidiuretic hormone in chemical composition and pharmacological action but is not detectable by the specific radioimmunoassay. On the other hand both vincristine and cyclophosphamide have the same effect on the hypothalamus and/or the hypothalamic-pituitary pathway causing ab-

normal secretion of ADH which continues to act paradoxically since hyponatremia would be expected to suppress ADH secretion. Cases have been recorded in which ADH was found at high levels by radioimmunoassay (7). Unfortunately ADH was not measured in our patient. In our child the drugs vincristine and/or cyclophosphamide must have caused the electrolyte disturbance since our patient was at the time in remission from her malignancy and the episode recurred when the drugs were given again.

As far as management is concerned some authors have in addition given a diuretic (2) e.g. furosemide (Lasix) and others have resorted to lithium therapy with encouraging results (1). In our child no maintenance therapy is needed at present.

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DISCUSSION

The accepted clinical and laboratory criteria for the diagnosis of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are the following: (a) hyponatraemia and hypo-osmolality of serum and extracellular fluid, (b) increased urinary sodium and hyperosmolality in relation to that of serum, (c) low serum chloride, (d) no clinical evidence of hypovolaemia, dehydration and edema, (e) no renal nor adrenal disease and (f) improvement of the above upon water restriction (4, 6, 7).

Our child probably represents a case of SIADH since she satisfies all of the above criteria. Her severely depleted electrolytes were indeed not restored by intravenous administration of 0.9% NaCl solution and KCl they were corrected by simple restriction of fluids. On the other hand the absence of albuminuria, glucosuria, hyposthenuria and increased serum creatinine exclude renal dis-

ease hypopotassemia without polyuria would also tend to exclude adrenal disease.

Our child however had in addition uremia, low serum potassium (2.5 mmol/l) and metabolic alkalosis (CO_2 62.4 mmol/l and blood pH 7.55). Whether these represent an expansion of the syndrome itself or some other associated abnormalities of obscure nature cannot be stated at present. Uremia was not renal since it was transient and not accompanied with other renal findings. It was observed only during both episodes. Low serum potassium has been observed in leukemia and other early or advanced lymphocellular malignancies (4) as well as after prolonged antibiotic administration (5). Our child did not receive antibiotics and her malignancy was in definite remission at the time. Alkalosis has been said to occur secondary to low serum potassium as a compensatory mechanism or as others believe hypopotassemia is due to the alkalosis itself. What is however impressive is the disappearance of uremia, hypopotassemia and alkalosis upon water restriction and the subsequent remission of the syndrome. The clinical and laboratory features of this syndrome not only disappeared after water restriction but reappeared when the same chemotherapy was given again a few weeks later to be restored once more after water restriction. During the 2nd episode uremia, hypopotassemia and alkalosis were also present. ECG changes were attributed to adriamycin and not to hypopotassemia since the latter was corrected whereas the ECG changes persisted. As far as causation is concerned a multitude of factors have been incriminated. SIADH may be due to the tumour itself (4) or to some other pathological state. It is believed that tumour cells synthesize and secrete a substance that simulates antidiuretic hormone in chemical composition and pharmacologic action but is not detectable by the specific radioimmunoassay. On the other hand both vincristine and cyclophosphamide have the same effect on the hypothalamus and/or the hypothalamic-pituitary pathway causing ab-

normal secretion of ADH which continues to act paradoxically since hyponatremia would be expected to suppress ADH secretion. Cases have been recorded in which ADH was found at high levels by radioimmunoassay (7). Unfortunately ADH was not measured in our patient. In our child the drugs vincristine and/or cyclophosphamide must have caused the electrolyte disturbance since our patient was at the time in remission from her malignancy and the episode recurred when the drugs were given again.

As far as management is concerned some authors have in addition given a diuretic (2) e.g. furosemide (Lasix) and others have resorted to lithium therapy with encouraging results (1). In our child no maintenance therapy is needed at present.

REFERENCES

- 1 Baker R S, Hurley M R & Feldman W. Treatment of recurrent syndrome of inappropriate secretion of antidiuretic hormone with lithium. *J Pediatr* 90: 480 1977.

- 2 Cohen L F, Di Sant Agnese P A, Taylor A & Gill J R. The syndrome of inappropriate antidiuretic hormone secretion as a cause of hyponatremia in cystic fibrosis. *J Pediatr* 9: 574 1977.
- 3 Fine M N, Clarke R R & Shore N A. Hyponatremia and vincristine therapy. *Am J Dis Child* 117: 756 1966.
- 4 O'Regan M, Carson S, Chesney R W & Drummond K N. Electrolyte and acid-base disturbances in the management of leukemia. *Blood* 49: 345 1977.
- 5 Stapleton F H, Nelson B, Vats T S & Linshaw M A. Hypokalemia associated with antibiotic treatment. *Am J Dis Child* 130: 1104 1976.
- 6 Steele T H, Serpick A A & Block J B. Antidiuretic response to cyclophosphamide in man. *J Pharmacol Exper Ther* 185: 745 1973.
- 7 Stuart M J, Cuaso C, Miller M & Oski F A. Syndrome of recurrent increased secretion of antidiuretic hormone following multiple doses of vincristine. *Blood* 45: 315 1975.
- 8 Weinberg J A, Weitzman R E, Zakandjian B & Leake M D. Inappropriate secretion of antidiuretic hormone in a premature infant. *J Pediatr* 90: 111 1977.

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CASE REPORT

SPONTANEOUS PERFORATION OF THE COMMON BILE DUCT

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ABSTRACT Enell H, Cavell B and Malmfors G (Departments of Paediatrics and Paediatric Surgery, University Hospital Lund, Sweden). Spontaneous perforation of the common bile duct. *Acta Paediatr Scand* 68: 625-1979. — An 18-week-old baby boy suffered an episode of acute abdominal symptoms followed by a silent period with mild obstructive jaundice, abdominal distension and failure to thrive. During the clinical work-up he deteriorated suddenly with progressive abdominal distension and ascites. At laparotomy a perforation of the common bile duct with bile peritonitis was found. Spontaneous perforation of the common bile duct is seldom listed as a cause of obstructive jaundice. The clinical picture is characteristic. Awareness of this diagnosis may help to avoid time consuming and unnecessary investigations and to lead to surgical treatment in good time.

KEY WORDS Obstructive jaundice, ascites, bile peritonitis, infancy.

Spontaneous perforation of the common bile duct is seldom discussed as a cause of obstructive jaundice in the paediatric literature (6-7). Lilly et al (5) however state that this disease is second only to biliary atresia as a cause of surgical jaundice in the newborn. Until 1974 about 50 cases had been reported, most of them in the surgical literature (5). The aim of this paper is to draw attention to this disease and to report a case with a protracted course.

CASE HISTORY

An 18-week-old boy was referred to a local hospital because of abdominal pains, irritability and anorexia. Pregnancy, delivery and neonatal period had been uneventful. On admission the abdomen was distended and tender. The stools were pale and the urine dark. The emergency examination excluded an intestinal obstruction.

The initial symptoms subsided gradually and the boy was referred 7 weeks later to the Paediatric Department, University Hospital. His general condition was then fairly good. Physical examination revealed a normally developed, slightly jaundiced baby with moderate abdominal distension. During the following weeks his general clinical condition further improved and his appetite returned. His weight however was stationary and the stools were still light-coloured.

Laboratory investigations showed a conjugated hyper-

bilirubinemia around $50 \mu\text{mol/l}$. Alkaline phosphatases $18 \mu\text{katal/l}$, glutamyltransferase $4.8 \mu\text{katal/l}$, aminotransferases normal, prothrombin time 32 s, urobilinogen/urine negative. Serum proteins including immunoglobulins were normal. Investigations for metabolic, endocrine or infectious causes of the illness turned out normal.

Abdominal plain films taken early in the course showed slightly dilated bowels. A barium enema of the colon and an intravenous pyelography gave normal results.

A choledochal cyst was suspected as a cause of the boy's stationary and mild cholestasis. At intravenous cholangiography the gallbladder appeared and emptied normally but the bile ducts were not visualized. In order to exclude an expansivity close to the bile duct causing intermittent obstruction, ultrasonic echogram and X-ray of the stomach and upper small intestine were performed. The patient deteriorated quickly, however, with marked abdominal distension and signs of ascites. The X-ray showed wide intestinal loops, slow passage and ascites, but no signs of tumour displacing the viscera. The ultrasonic echogram was negative. The boy was referred for emergency exploratory laparotomy in the department of paediatric surgery.

On admission to this department the patient was in a rather poor general condition. Preoperatively he was given proper amounts of fluid, electrolytes and plasma. At laparotomy nearly one litre of bile stained fluid was emptied from the abdominal cavity, before the bile ducts were exposed. An attempt to make a cholangiogram through the gallbladder was not successful. The choledochal duct was dissected and choledochotomy was performed. The lumen of the choledochal duct was normal whereas the external diameter was more than 1 cm due to a peritonitic swelling. A catheter was passed to the

CASE REPORT

SCLERODERMA WITH MASSIVE REGIONAL LYMPHADENOPATHY

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ABSTRACT Tangsrud S E, Skyberg D and Larsen Tove Eeg (Department of Paediatrics, Sentralsjukehuset i Aust Agder, Arendal, Norway and the Department of Pathology, Rikshospitalet, Oslo, Norway). Case Report: Scleroderma with massive regional lymphadenopathy. *Acta Paediatr Scand* 68: 627-1979. —Scleroderma in a two-year-old boy with gross enlargement of the right inguinal lymph nodes as an early sign is reported. Repeated lymph node biopsies revealed non-characteristic reactive changes and hyperplasia but eventually histological examinations of skin and muscle from the right leg were diagnostic. The effect of one year of D-penicillamine therapy is briefly mentioned.

KEY WORDS Scleroderma, lymphadenopathy, D-penicillamine.

Scleroderma is a connective tissue disease of unknown etiology. It is rare in childhood and may involve the skin, gastrointestinal tract, heart, lung and kidney. There seems to be no sharp distinction between the focal form (morphoea) and the diffuse systemic disease (2, 3). Raynaud's phenomenon, positive antinuclear factor, LE cells and raised erythrocyte sedimentation rate are associated with poor prognosis (2).

We present a case with regional lymphadenopathy as the initial sign.

CASE REPORT

A previously healthy, year-old boy was admitted because of enlargement of the inguinal lymph nodes and episodic pain in the right leg.

The parents noticed the swelling in the groin 8 weeks prior to admission and 3 weeks later the mother also noticed skin changes in the right leg. Initially there were small red scaling patches on the skin. Later the patches became stretched and band-like along the length of the leg.

On admission his general condition was good. General lymphadenopathy was absent. Liver and spleen were not increased in size. There was marked enlargement of the right inguinal lymph nodes, which were firm, tender and adherent to each other and to deeper structures. Areas of increased pigmentation as well as areas with lack of

pigmentation were noted mainly in the gluteal region, perineum and the medial surface of the right leg. The skin was thin, atrophic and partly adherent to the underlying structures which were indurated. Muscular wasting of the right calf and a 10-15° contracture in the right knee were present. A telangiectatic area about 5 mm in diameter, which had appeared during the first weeks of illness, was noted on the right cheek. Due to pain related to the lymph nodes, he was almost unable to walk.

Investigations

Hb 116 g/l, ESR 19 mm/hr, WBC $10.0 \times 10^9/l$ with lymphocytic dominance, platelets $400 \times 10^9/l$. Serum ASAT 103 U/l (normal range 10-40 U/l), Serum ALAT 78 U/l (normal range 10-40 U/l), Gamma GT, OCT, LDH, CPK and creatinine were all normal.

The serum fractions of IgA, IgM and IgE were normal while IgG was slightly elevated (16 g/l). Serological tests for ornithosis, toxoplasmosis, salmonellosis, brucellosis, streptococcosis, mononucleosis and Epstein Barr virus were negative. A PPD test was negative. A negative LE preparation, a positive test for antinuclear antibodies (titre 3+) and a strongly positive anti-DNA denaturated factor were found. Serum complement C_3 and C_4 , ECG and respiratory function tests were within normal limits. X-ray examinations of chest, oesophagus, stomach and the skeleton were normal.

A lymph node biopsy from the enlarged glands in the right inguinal region was performed the day after admission. Three weeks afterwards another lymph node biopsy from the same region was performed, together with a skin and muscle biopsy from the affected area of the right calf. Both lymph node biopsies showed reactive changes and hyperplasia. A skin biopsy from the lower extremity

duodenum proving passage. A leak was found a few millimetres distal of the cystic duct on the right lateral surface of the choledochal duct. The leak was oversewn and a choledochal drain was left in place. The area was thoroughly drained.

The postoperative course was quite uneventful. A cholangiogram on the seventh day was normal. A few months post-operatively the child was healthy with normal liver function tests.

DISCUSSION

The clinical features presented by our patient agree well with those reported as characteristic in the literature (2-4, 5). The infants usually present with symptoms at the age of one week to 3 months. Initial signs include mild fluctuating jaundice, pale or acholic stools, dark urine, irritability, vomiting, and failure to thrive. There is a progressive abdominal distension which is sometimes accompanied by bile staining of hydroceles, inguinal herniae or of the abdominal wall. The mild hyperbilirubinemia together with acholic stools indicate the correct diagnosis. The normal immunotransferases are helpful in differentiating from hepatitis. The ¹³¹I Rose Bengal test may establish the presence of a biliary duct (1, 2).

A protracted course of the disease is observed in the case here reported, seems to be the most usual one, but an acute mode of presentation is sometimes seen (4).

The symptoms and the time of onset are helpful in distinguishing the disease from other causes of obstructive jaundice in infancy such as biliary atresia and choledochal cyst (3).

It has been suggested that spontaneous perforation of the common bile duct and choledochal cyst are different manifestations of a common developmental aberration in ductal embryogenesis (5).

In many cases of spontaneous perforation of the common bile duct the operative finding is

an inflammatory sac which has been misinterpreted as a ruptured choledochal cyst. This may lead to an inadequate operative management with lethal outcome (5). We think that such a sac was initially formed in our patient after which his condition improved. The sac then ruptured and gave rise to the alarming signs of bile peritonitis.

Different operative procedures have been suggested from simple drainage to choledochal resections with hepaticoenteric anastomosis. It seems as the simple drainage procedure is the method of choice (4, 5).

Spontaneous perforation of the common bile duct is a rare disease. On the other hand an awareness of the condition is important as immediate surgical treatment is indicated.

REFERENCES

- 1 Hansen R, Wisnitch R, DeVries P & Sunshine P. Bile ascites in infancy. Diagnosis with ¹³¹I Rose Bengal. *J Pediatr* 94: 719, 1974.
- 2 Howland E, Johnston D & Mowat A. Spontaneous perforation of common bile duct in infants. *Arch Dis Child* 51: 993, 1976.
- 3 Kobayashi A & Ohbe Y. Choledochal cyst in infancy and childhood. *Arch Dis Child* 52: 121, 1977.
- 4 Lees W & Mitchell J. Bile peritonitis in infancy. *Arch Dis Child* 41: 189, 1966.
- 5 Lilly J, Weintraub W & Altman P. Spontaneous perforation of the extrahepatic bile ducts and bile peritonitis in infancy. *Surveys* 75: 664, 1974.
- 6 Mowat A. Persistent neonatal jaundice. In J T Harris (ed.) *Essentials of Pediatric Gastroenterology*. Churchill Livingstone, Edinburgh, London and New York, 1977, p. 766.
- 7 Saks Kortsik A. Management of young infants presenting with direct reacting hyperbilirubinemia. *Pediatr Clin N Am* 21: 777, 1974.

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CASE REPORT

SCLERODERMA WITH MASSIVE REGIONAL LYMPHADENOPATHY

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From the Department of Paediatrics, Sentralsjukehuset i Aust Agder, Arendal, Norway, and the Department of Pathology, Rikshospitalet, Oslo, Norway

ABSTRACT Tangsrud S ■ Skyberg D and Larsen Tove Egg (Department of Paediatrics, Sentralsjukehuset i Aust Agder, Arendal, Norway, and the Department of Pathology, Rikshospitalet, Oslo, Norway) Case Report Scleroderma with massive regional lymphadenopathy. *Acta Paediatr Scand* 68: 627-1979. Scleroderma in a two-year-old boy with gross enlargement of the right inguinal lymph nodes as an early sign is reported. Repeated lymph node biopsies revealed non-characteristic reactive changes and hyperplasia, but eventually histological examinations of skin and muscle from the right leg were diagnostic. The effect of one year of D-penicillamine therapy is briefly mentioned.

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Investigations

Hb 116 g/l, ESR 25 mm/hr, WBC $19.0 \times 10^9/l$ with lymphocytic dominance, platelets $110 \times 10^9/l$. Serum ASAT 103 U/l (normal range 10-40 U/l), Serum ALAT 78 U/l (normal range 10-40 U/l), Gamma GT, OCT, LDH, CPK and creatinine were all normal.

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REFERENCES

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- 2 Howard E, Johnston D & Mowat A. Spontaneous perforation of common bile duct in infancy. *Arch Dis Child* 51: 883, 1976.
- 3 Kobayashi A & Ohbe Y. Choledochal cyst in infancy and childhood. *Arch Dis Child* 53: 171, 1977.
- 4 Lees W & Mitchell J. Bile peritonitis in infancy. *Arch Dis Child* 41: 189, 1966.
- 5 Lilly J, Weintraub W & Altman P. Spontaneous perforation of the extrahepatic bile ducts and bile peritonitis in infancy. *Surveys* 75: 664, 1974.
- 6 Mowat A. Persistent neonatal jaundice. In J T Harris (ed.) *Essentials of Pediatric Gastroenterology*. Churchill Livingstone, Edinburgh, London and New York, 1977, p. 266.
- 7 Siskind S. Management of young infants presenting with direct reacting hyperbilirubinaemia. *Pediatr Clin N Am* 21: 777, 1974.

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CASE REPORT

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From the Department of Paediatrics Sentralsjukehuset i Aust Agder Arendal Norway and
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The serum fractions of IgA IgM and IgE were normal while IgG was slightly elevated (16 g/l) Serological tests for ornithosis toxoplasmosis salmonellosis brucellosis streptococcosis mononucleosis and Epstein Barr virus were negative A PPD test was negative A negative LE preparation a positive test for antinuclear antibodies (titre 32) and a strongly positive anti DNA denaturated factor were found Serum complement C and C₃ ECG and respiratory function tests were within normal limits X ray examinations of chest oesophagus stomach and the skeleton were normal

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Fig. 1 The patient's right leg, after 6 months of illness

showed dermal fibrosis and fibrotic subcutaneous septa. In the lower dermis and in the subcutaneous septa there were dilated blood vessels and a perivascular as well as diffuse lymphoplasmocytic infiltration. This picture was compatible with scleroderma.

Treatment and subsequent course

Several authors have reported encouraging results with the use of D-penicillamine in treatment of childhood scleroderma (4-5). Our patient received 150 mg D-penicillamine every second day. During the observation time of one year no side effects were observed.

In addition he was given physiotherapy. The skin has gradually improved and the induration of the deeper structures has become less firm. The boy has complained of dysphagia and a barium X-ray examination of the oesophagus revealed a probable dysfunction of the distal part of this organ. His general condition is good and ESR has normalized.

DISCUSSION

Reports on childhood scleroderma are few. In some larger series (1-3) none of the patients were reported to have lymph node enlargement. Dubich et al (1) found skin changes as presenting sign in 100% in a group of twelve children. Joint pains, contractures and Raynaud phenomenon are also frequent (1-3).

Because of serological and clinical overlap

specific diagnosis in childhood connective tissue diseases is often difficult. Differential diagnoses include dermatomyositis, juvenile rheumatoid arthritis and SLE.

Thomson & Milne (5) reported a girl who when 12 years old presented with enlarged neck lymph nodes and a skin rash resembling erythema multiforme. A lymph node biopsy showed no specific changes. She developed arthritis and 4 years later scleroderma was diagnosed by a skin and muscle biopsy.

The present case and the case reported by Thomson & Milne (5) draw attention to the fact that lymph node enlargement may be a prominent and early sign in childhood scleroderma and may precede cutaneous manifestations. Biopsies of the affected lymph nodes are of little diagnostic value. In the series of Dubich et al (1) the average time between appearance of symptoms and the final diagnosis was 2 years.

Early skin and muscle biopsies should therefore be considered in any obscure cases of childhood connective tissue disease with skin or lymph node involvement. The course in our patient may support the value of D-penicillamine in the treatment of scleroderma (4-5).

REFERENCES

1. Dubich I, Sullivan D & Cassidy J T. Scleroderma in the child. *Pediatr* 85: 677-1974.
2. Goel K M & Shanks R A. Scleroderma in childhood. Report of five cases. *Arch Dis Child* 49: 86-1974.
3. Kays H, Hanson V & Patrick J. Scleroderma in childhood. *J Pediatr* 69: 243-1966.
4. Moynahan E J. Penicillamine in the treatment of morphea and keloid in children. *Postgrad Med J* 49: 39-1974.
5. Thomson J & Milne J A. Two years of penicillamine for progressive systemic sclerosis. A case report. *Postgrad Med J* 50: 36-1974.

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CASE REPORT

NONKETOTIC HYPERGLYCINEMIA

Clinical Biochemical and Therapeutic Aspects

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ABSTRACT Kolvraa S Brandt N J and Christensen E (Section of Clinical Genetics Department of Paediatrics and Department of Neonatology University of Copenhagen Rigshospitalet Copenhagen Denmark) Nonketotic hyperglycinemia Clinical biochemical and therapeutic aspects *Acta Paediatr Scand* 68 629 1979 —A patient exhibiting progressive cerebral depression from the first days of life is described The diagnosis of nonketotic hyperglycinemia was established on the typical clinical presentation elevated glycine concentrations in body fluids and diminished glycine cleavage activity in liver tissue A series of therapeutic trials including strychnine treatment was tried on this patient without apparent effect on the clinical course

KEY WORDS Nonketotic hyperglycinemia glycine cleavage activity strychnine treatment

Hyperglycinemia was first described as a syndrome by Childs et al (3) It is now possible on clinical and biochemical criteria to differentiate this syndrome into several distinct entities namely the disease presented in this paper nonketotic hyperglycinemia the ketotic forms propionic acidemia (1) methylmalonic acidemia (10) and β ketothiolase deficiency (5) and the newly published D-glycemic acidemia (2 7)

Nonketotic hyperglycinemia is clinically characterized by neonatal onset of hypotonia lethargy and seizures followed by severe mental retardation Biochemically it is characterized by elevated glycine concentrations in blood urine and spinal fluid and diminished activity of the glycine cleavage system in liver and brain (12)

Many therapeutic attempts have been tried to relieve the symptoms of nonketotic hyperglycinemia Most of these have aimed at lowering the glycine concentration in serum either by diet by administering compounds that form easily excretable conjugates with

glycine or by donating C₁ fragments (11) Recently a more direct attempt to lower spinal fluid glycine has been tried by Krieger et al (6) namely insertion of a ventricular shunt Strychnine treatment has also been tried (4) This is based on the observation that the effect of glycine on synaptic transmission is contrary to the effect of strychnine especially on spinal centres (15)

The present communication describes the clinical and biochemical presentation of a case of nonketotic hyperglycinemia together with the therapeutic attempts that we have performed

CASE REPORT

The patient a boy is the first child of nonconsanguineous parents The mother has in a previous marriage given birth to a normal boy Pregnancy and delivery was uneventful Birth weight 3600 g The child appeared normal at birth but from the second day of life he became increasingly listless From the fifth day insufficient respiration occurred which caused the patient to be transferred to the University Hospital Examination on admission revealed a severely hypotonic child with no spontaneous movements and no reactions to stimuli Artificial

Table 1 Quantitative determination of serum and urine amino acids

	Serum ($\mu\text{mol/l}$)		Urine ($\mu\text{mol}/24 \text{ hrs}$)	
	Patient	Range in controls	Patient	Range in controls ^a
Aspartic acid	91	25-72	19	33-181
Threonine	149	69-125	47	4-125
Serine	435	103-212	102	5-241
Asparagine	69	37-51	59	5-108
Glutamic acid/glutamine	497	309-422	46	46-277
Proline	246	119-233	Trace	Trace
Glycine	1226	160-782	4894	166-495
Alanine	187	272-375	80	45-231
Valine	217	121-206	5	11-22
Cystine	31	24-38	9	Trace-11
Methionine	29	12-18	Trace	Trace-17
Isoleucine	85	30-57	Trace	Trace-9
Leucine	155	64-104	Trace	Trace-19
Tyrosine	87	37-52	29	Trace-71
Phenylalanine	54	35-54	8	Trace-46
Ornithine	146	62-92	Trace	Trace-79
Lysine	166	114-201	30	19-75
Histidine	97	59-88	211	117-686
Arginine	88	61-129	4	Trace-10

^a 4 children 1-6 years old (2)

^b 8 children 1-6 years old (2)

Trace <2 $\mu\text{mol}/24 \text{ hrs}$

respiration had to be instituted immediately after admission

Laboratory investigations on admission Routine laboratory investigations including haemoglobin, white blood cell count, thrombocytes, blood glucose and electrolytes were normal. The acid-base status showed a moderate respiratory acidosis. No ketosis was present. Amino acid analysis of serum and urine both demonstrated marked elevation of glycine (Table 1). Spinal fluid was obtained in a period where the serum glycine concentration was stabilized around 500 $\mu\text{mol/l}$. At the day of sampling serum glycine was 524 $\mu\text{mol/l}$ and spinal fluid glycine 187 $\mu\text{mol/l}$.

Further clinical course Intensive glycine depleting therapy was given from the 8th to the 28th day of life (see Initial therapeutic attempts). This resulted in re-establishment of spontaneous respiration, but apart from this no clinical improvement could be demonstrated. From the 10th day of life seizures resembling infantile myoclonic jerks occurred, which necessitated antiepileptic treatment. EEG at this time showed severe hypsarrhythmia. The further clinical course has been characterized by a convulsive disorder difficult to control (in spite of treatment with Clonazepam (Rivotril®) 1.2 mg/day and Phenobarbital 30 mg/day) and severe mental retardation. Physically he has developed normally. Weight, length and head circumference have constantly been within the tenth percentile.

MATERIAL AND METHODS

Amino acids and organic acids were measured as previously described (7).

Glycine cleavage activity At six months of age a surgical liver biopsy was obtained from the patient. The biopsy was immediately frozen and kept at -70°C . Glycine cleavage activity was measured according to Sato et al. (13). 25 mg liver tissue was homogenized and incubated with ^{14}C glycine. Reaction mixture: 4.75 μmol glycine (specific activity 0.8 mCi/mmol), 0.5 μmol trihydrofolic acid, 0.8 μmol pyridoxal phosphate, 0.9 μmol dithiothreitol, 0.8 μmol nicotinamide adenine dinucleotide, 75 μmol Tris HCl (pH=8.1), 3 mg protein, total volume 1.0 ml.

After incubation for one hour the reaction was stopped by adding 0.2 ml sulphuric acid (5 mol/l). The liberated CO_2 was trapped on Protozol® scintillation fluid added and ^{14}C activity counted in a liquid scintillation counter. Activity was expressed in nmol CO_2 produced/mg protein/hour. Protein was determined according to Lowry (9).

RESULTS

Amino acids The amino acid examinations demonstrated an isolated accumulation of glycine in body fluids both on admission (Table 1) and on several later occasions when amino acids were investigated in connection with therapeutic attempts. The ratio of spinal fluid glycine to serum glycine was 0.36.

Organic acids Low molecular weight organic acids in the urine were examined by gas

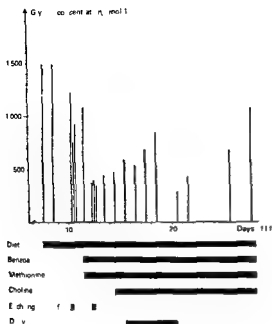


Fig 1 Course of serum glycine concentration during the initial therapeutic attempts

chromatography as trimethylsilyl derivatives and as free volatile acids. No accumulation of propionic acid, methylmalonic acid, 2-methyl-3-hydroxybutyric acid, 2-methyl-3-oxo-butanoic acid or D-glycemic acid could be demonstrated.

Glycine cleavage. In preliminary experiments it was ascertained that the glycine cleavage reaction rate increased linearly with the protein concentration and that the reac-

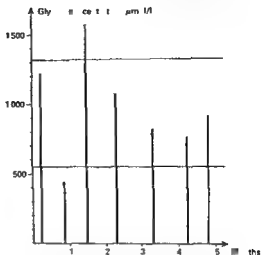


Fig 2 Spontaneous fluctuation in serum glycine concentration during a 5 month period. The horizontal lines indicate range defined as mean serum glycine concentration \pm 2 SD.

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Tissue samples from two autopsic livers obtained 8 and 12 hours post mortem and one surgical liver biopsy were used as controls. The glycine cleavage activities found are shown in Table 2 together with the diagnoses of the children.

THERAPEUTIC ATTEMPTS

Initial therapeutic attempts. The diagnosis nonketotic hyperglycinemia was established on the eighth day of life and the following therapeutic steps were taken (Fig 1). Breast milk was substituted by a mixture containing per liter: 25 g glycine, serine and proline free mixture of amino acids, salts and vitamins (Maizena Haus Hamburg), 30 g glucose, 30 g fructose, 350 ml Intralipid® 200 ml per day of this mixture was given initially, gradually increasing to 700 ml per day on day 25. In addition the child received the usual vitamin supplement for his age. On the first day of therapy an exchange transfusion was performed and medication was started with benzoic acid 100 mg/kg/day and methionine 150 mg/kg/day.

Two days later another exchange transfusion was performed and treatment with choline bitartrate 85 mg/kg/day was instituted. Finally from the 16th day to the 70th day of life a continuous peritoneal dialysis was performed. During this treatment the general condition improved only slightly, although at the end of the period the patient was capable of unassisted breathing. He was however still extremely hypotonic and showed only very weak reactions to stimuli.

Table 2 Glycine cleavage activity in liver tissue

	Diagnosis	Glycine cleavage (nmol/mg protein/hour)
Patient	Nonketotic hyperglycinemia	0.095
Control A	Mild hepatic stasis (surgical biopsy)	0.570
Control B	Cong. heart disease (autopsic biopsy)	0.970
Control C	Cong. heart disease (autopsic biopsy)	1.040

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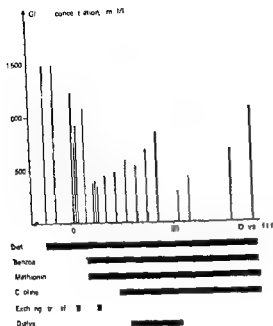


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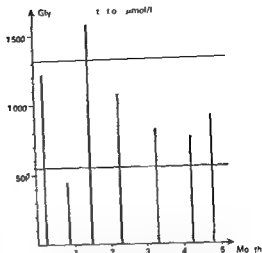


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Later medication was tried with compounds that react with glycine, followed by elimination of the conjugate, such as benzoic acid and acetylsalicylic acid. In recent years still another approach has been proposed, namely to donate C_1 fragments. This is based on the assumption that there is a shortage of C_1 fragments due to deficiency of the glycine cleavage activity. The C_1 fragments should then diminish the glycine pool by transforming glycine to serine. Of possible C_1 -donors methionine, choline and leucovorine have been tried. Varying results have been reported, but generally no effect on the clinical course and only weak and temporary effects on the serum glycine concentration have been obtained with these treatments.

Because of the early diagnosis in our patient we decided to make a massive therapeutic effort in the neonatal period in order to see if mental retardation could be prevented. As shown in Table 1, rapid lowering of serum glycine was obtained. It seems, however, that the effect was achieved only by exchange transfusion and dialysis, since serum glycine concentrations immediately started to rise after termination of these, finally reaching pretreatment values in spite of diet and continuous treatment with benzoate, methionine and choline.

It is therefore likely that neither benzoate nor methionine nor choline have sufficient glycine-depleting effect to be of therapeutic benefit. This impression was further confirmed in the systematic trials, where no significant effect could be demonstrated after treatment for 10 days with any of these compounds.

A major reason for the almost total lack of

clinical response on the normalisation of serum glycine concentration induced by exchange transfusion and dialysis is most likely that the symptoms are not caused by the elevated serum glycine. They probably result from a raised spinal fluid glycine concentration, especially in the synaptical cleft, where glycine exerts its neurophysiological effect (14). The concentration here is normally extremely low (about $25 \mu\text{mol/l}$) due to both a high and a low affinity transport system, which removes glycine by transporting it against gradients both into the cells and into the blood stream (8). The spinal fluid glycine concentration in nonketotic hyperglycinemia is disproportionately high. This is probably due to the lack of cerebral glycine cleavage activity, which causes intracerebral and spinal fluid glycine accumulation. When the spinal fluid glycine concentration reaches a certain level it results in saturation of the transport systems, with further rapid raising of the spinal fluid glycine concentration as a result. When the patient has reached this state, lowering the serum glycine concentration will probably be almost completely ineffective, since only a diminution of the cerebrospinal glycine pool, extensive enough to create a concentration gradient direct away from the cerebrospinal room, would re-establish the transport systems within reasonable time.

Therapeutic trials in nonketotic hyperglycinemia have therefore, in the last few years, been centered more on the cerebral mechanism than on lowering the serum concentration. The first attempts along these lines were made by Krieger et al. (6), who implanted a ventricular shunt in order to wash out the spinal fluid glycine more effectively. Unfortunately the patient was only followed 5 weeks post-operatively, so the effect of this treatment is uncertain. More recently Gitzelman et al. (4) have tried strychnine treatment with excellent effect on muscular tonus. These promising results prompted us to try this treatment in our patient. We could not, however, demonstrate any effect, but this might be due to the fact

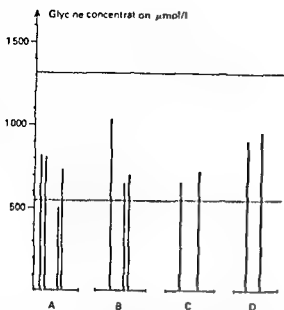


Fig. 3 Serum glycine concentration during therapeutic attempts (A) benzoic acid 200 mg/kg/day (B) methionine 150 mg/kg/day (C) choline 85 mg/kg/day (D) benzoic acid 200 mg/kg/day + methionine 150 mg/kg/day. Prior to each therapeutic attempt the serum glycine concentration was measured (first set of bars). Treatment was then given for 10 days. On the 1st day of treatment the serum glycine concentration was measured again (second set of bars). The horizontal lines indicate range during periods without treatment.

The effect on the glycine concentration in serum of the various forms of treatment is shown in Fig. 1.

Long-term spontaneous fluctuations in glycine concentrations. When it was apparent that massive mental retardation was inevitable, all treatments were withdrawn and benzoic acid, methionine and choline were tried separately for ten days each to further elucidate their effects on the hyperglycinemia.

Prior to such a systematic investigation it was considered necessary to clarify the magnitude of spontaneous fluctuations in the serum glycine concentration since these might bias the results.

In order to achieve this, serum samples collected more than 3 weeks after termination of any therapy were obtained over a period of five months. The glycine concentrations of these samples are shown in Fig. 2 (mean glycine concentration \pm S.D. 921 ± 399 μ mol/l).

Therapeutic attempts with benzoate, methionine and choline. All therapeutic attempts were performed in a similar manner when the patient was 1–2 years old. Initially the serum glycine concentration was measured either as a single or as a double determination on two consecutive days. Treatment was given for ten days with either benzoate 200 mg/kg/day, methionine 150 mg/kg/day, choline 85 mg/kg/day or the combination of benzoate 200 mg/kg/day and methionine 150 mg/kg/day. At the end of each 10-day period serum glycine concentration was again measured either as a single or as a double determination. The results of these investigations are shown in Fig. 3.

A certain effect of benzoate (3A) and methionine (3B) was apparent in effect, however, that could not be reproduced when giving the two compounds together (3D). The reason for this inconsistency is most likely to be the substantial intra-individual fluctuations in serum glycine concentration that are pictured in Fig. 2. Medication with choline had no effect on the serum glycine concentration (3C).

No clinical improvement could be demonstrated during any of these trials, neither on tonus nor on number of seizures.

Strychnine treatment. Treatment with strychnine nitrate 0.3 mg/kg/day was tried when the patient was 6 months old, but neither improved tonus nor diminished number of seizures was achieved.

DISCUSSION

The clinical picture with elevated concentrations of glycine in body fluids and absence of increased excretion of organic acids confirmed the diagnosis nonketotic hyperglycinemia. It was further supported by the demonstration of diminished glycine cleavage activity in liver tissue. As shown in Table 1, a higher activity was found in autopsy material compared to the surgical liver biopsy. The significance of this is at present unclear, but since the patient possessed only 20% of the activity in fresh liver tissue, we believe it is safe to conclude that diminished glycine cleavage activity is present.

Additional support for the diagnosis was achieved by the finding that the ratio of glycine concentration in spinal fluid to that in serum was 0.36. This ratio is normally 0.02. As pointed out by Perry et al. (12), elevation of this ratio may indicate nonketotic hyperglycinemia, where ratios about 0.2 have been found.

The basic defect in this disorder is considered to be the diminished glycine cleavage activity, which has been demonstrated in all cases so far investigated. In liver tissue activities between 10 and 50% of normal have been found (12). In brain tissue the glycine cleavage activity is normally about 50% of the liver activity, while in patients with nonketotic hyperglycinemia no activity could be demonstrated (12).

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that the treatment was initiated when he was 26 months old and already severely brain damaged

ACKNOWLEDGEMENT

This work was supported by the Danish Medical Research Council

REFERENCES

- 1 Ando T & Nyhan W L Propionic acidemia and the ketotic hyperglycinemia syndrome In W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
- 2 Brindt N J Rasmussen K Brindt S Kolvraa S & Schönheyder F D-glycine acidemia and nonketotic hyperglycinemia *Acta Paediatr Scand* 65 17 1976
- 3 Childs B Nyhan W L Borden M Bard L & Cook H F Idiopathic hyperglycinemia and hyperglycinuria A new disorder of amino acid metabolism *Pediatrics* 27 522 1961
- 4 Gitzelmann R Cuenod M Otten A Steinmann B & Dumermuth G Nonketotic hyperglycinemia treated with strychnine *Pediatr Res* 11 1016 1977
- 5 Hillman R E & Keating J P β ketothiolase deficiency as a cause of the ketotic hyperglycinemia syndrome *Pediatrics* 53 271 1974
- 6 Kneiger J Winbium E S & Eisenbrey A H Cerebro-spinal fluid glycine in nonketotic hyperglycinemia Effect of treatment with sodium benzoate and a ventricular shunt *Metabolism* 76 517 1977
- 7 Kolvraa S Rasmussen K & Brindt N J D-glycine acidemia biochemical studies of a new syndrome *Pediatr Res* 10 825 1976

- 8 Laytha A Amino acid transport in the brain *in vivo* and *in vitro* In G E W Wolstenholm & D W Fitzsimmons (eds) *Aromatic amino acids in the brain* Ciba Foundation Symp 22 p 25 Elsevier New York 1974
- 9 Lowry O H Rosebrough N J Farr A L & Randall H J *J Biol Chem* 193 265 1951
- 10 Morrow G Methylmalonic acidemia In W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
- 11 Nyhan W L Nonketotic hyperglycinemia In W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
- 12 Perry T L Urquart N MacLean J Evans M E Hansen S Davidson A G F Applegarth D A MacLeod P J & Lock J E Nonketotic hyperglycinemia *N Engl J Med* 292 1769 1975
- 13 Sato T Kochi H Sato N & Kakuchi G Glycine metabolism by rat liver mitochondria *J Biochem* 65 77 1969
- 14 Snyder S H The glycine synaptic receptor in the mammalian central nervous system *Br J Pharmacol* 53 473 1975
- 15 Young A B & Snyder S H Strychnine binding associated with glycine receptors of the central nervous system *Proc Nat Acad Sci USA* 70 7817 1973

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CASE REPORT

DELETION OF THE LONG ARM OF CHROMOSOME 11

A Clinical Entity

I FELDING and F MITELMAN

From the Department of Paediatrics Central Hospital Helsingborg and Department of Clinical Genetics Lund University Hospital Lund, Sweden

ABSTRACT Felding I and Mitelman F (Department of Paediatrics Central Hospital Helsingborg and Department of Clinical Genetics Lund University Hospital Lund, Sweden) Deletion of the long arm of chromosome No. 11. A clinical entity. *Acta Paediatr Scand* 68: 635, 1979. — A deletion of the long arm of chromosome No. 11, an aberration undetectable in conventional chromosome staining, was identified with Giemsa banding in a female infant with multiple congenital anomalies. A survey is given of the clinical findings in the few cases so far reported.

KEY WORDS Chromosome aberration, banding technique, congenital anomalies.

The introduction of chromosome banding techniques has been of great importance for the delineation of new clinical syndromes. The purpose of this paper is to describe the clinical findings in patients with a structural chromosome aberration which has escaped detection with the conventional staining technique.



Fig 1 Appearance of the newborn baby



Fig 2 Appearance of the patient at the age of 4 months

that the treatment was initiated when he was 26 months old and already severely brain damaged

ACKNOWLEDGEMENT

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REFERENCES

- 1 Ando T & Nyhan W L Propionic acidemia and the ketotic hyperglycinemia syndrome In W L Nyhan (ed) *Heritable disorders of amino acid metabolism* John Wiley & Sons New York 1974
- 2 Brindt N J Rasmussen K Brindt S Kolvraa S & Schonheyder F D-glycine acidemia and nonketotic hyperglycinemia *Acta Paediatr Scand* 65 17 1976
- 3 Childs B Nyhan W L Borden M Bard E & Cook R E Idiopathic hyperglycinemia and hyperglycinuria A new disorder of amino acid metabolism *Pediatrics* 77 422 1961
- 4 Gitzelmann R Cuenod M Otten A Steinmann B & Dumermuth G Nonketotic hyperglycinemia treated with strychnine *Pediatr Res* 11 1016 1977
- 5 Hillman R E & Keating J P β ketothiolase deficiency as a cause of the ketotic hyperglycinemia syndrome *Pediatrics* 53 221 1974
- 6 Krieger J Winbaum E S & Eisenberg A H Cerebro-spinal fluid glycine in nonketotic hyperglycinemia Effect of treatment with sodium benzoate and a ventricular shunt *Metabolism* 26 517 1977
- 7 Kolvraa S Rasmussen K & Brindt N J D-glycine acidemia biochemical studies of a new syndrome *Pediatr Res* 10 825 1976

- 8 Lajtha A Amino acid transport in the brain in vivo and in vitro In G E W Wolstenholm & D W Fitzsimmons (eds) *Aromatic amino acids in the brain* Ciba Foundation Symp 22 p 25 Elsevier New York 1974
- 9 Lowry O H Rosebrough N J Farr A L & Randall R J *J Biol Chem* 193 65 1951
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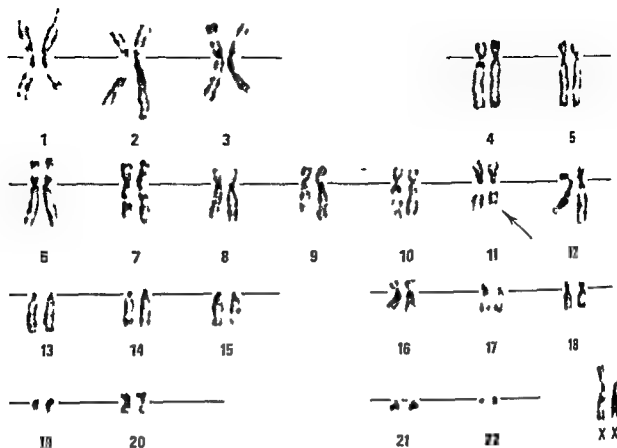


FIG. 3. Trypsin Giemsa banded karyotype showing a terminal deletion (arrow) of chromosome 11 del(11)(q22).

CASE REPORT

Clinical findings

Our patient, a girl born in 1975, was the second child of healthy unrelated parents, both 30 years old. The elder brother is healthy. There was no history of abortions, no family history of congenital defects and no exposure of parents or fetus to medical treatment, infection or radiation. After an uneventful pregnancy, spontaneous delivery occurred at 36 weeks gestation. Birth weight 1580 g, length 46 cm, small for gestational age. Apgar score at 1 and 5 min 8.

The placenta weighed 375 g and showed regressive changes, oedema and hemorrhage, some villi were hyalinized. There was no evidence of intrauterine infection measured by total IgM, not even after special separation for rubella and cytomegalovirus infection. Virus isolation of samples from mother and infant were negative as well as serologic tests for toxoplasmosis, rubella, cytomegalovirus and herpes simplex.

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Laboratory investigations

Blood counts normal, except for a transient thrombocytopenia. No vacuolated lymphocytes. Blood glucose, electrolytes, thyroid, liver and renal function tests normal.

Small for gestational age
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 Hand and foot deformities
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 Broad bridged nose
 Micrognathia
 Carp-shaped mouth
 Ear abnormalities
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 Hypertelorism
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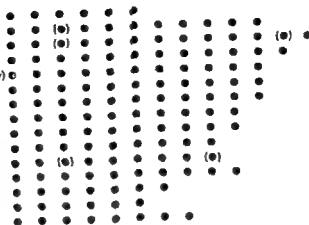


Fig 4 Major clinical symptoms in reported cases of 46 XX del(11) (q¹-q⁷) 13 ♀ & ♂

Routine urinary metabolic screening including amino acid urinary excretion normal Cerebrospinal fluid normal Lysosomal enzymes including β galactosidase β glucosidase α mannosidase α fucosidase and β hexosaminidase normal Electrocardiogram pattern of ventricular strain Electroencephalogram normal for age

Radiographic examination

Thin skull but otherwise normal bones Normal hands and feet and corresponding in age except for an ossified phalanx in the sixth toe and supernumerary fingers with out ossification Pelvis and hip-joints normal Chest X ray showed cardiac enlargement and dilated lung vessel Oesophagus and ventricle normal

DISCUSSION

So far 14 cases with a terminal deletion of the long arm of chromosome No 11 have been reported by Coco & Penchaszadeh (1) Engel et al (2) Faust et al (3) Frank & Riccardi (4) Jacobsen et al (5) Kaffé et al (6) Larson et al (7) Linarelli et al (8) Mulachy & Jenkyn (9) Turleau et al (10) and Zabel et al (11)

It might be of interest that females have accounted for 13 of the 15 cases with this deletion Only in one family was the deletion inherited through a translocation carrier parent (5) With the exception of one case (3) showing a more unspecific dysplastic appearance though loss of apparently the same amount of genetic material all other cases including our patient had the following main character

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- Coco R & Penchaszadeh V B Partial deficiency of long arm of chromosome No 11 *J Genet Hum* 25 43 1977
- Engel E Heirschberg C S Cassidy S H & McGee H J Chromosome 11 long arm partial deletion A new syndrome *Am J Med Defic* 80 473 1976
- Faust J Vogel W & Loring B A case with 46 XY del(11)(q¹) *Clin Genet* 6 90 1974
- Frank J & Riccardi V M The 11q syndrome *Hum Genet* 35 241 1977
- Jacobsen P Hauge M Renning en K Hobolth N Mikkelsen M & Philip J An (11-1) translocation in four generations with chromosome 11 abnormalities in the offspring *Hum Hered* 23 568 1973
- Kaffé S Hsu L Sachdev R Philips J & Hirschhorn K Partial deletion of long arm of chromosome 11 del(11)(q³) *Clin Genet* 12 373 1977
- Larson S A Yeatman S W & Riccardi V M Deletion of 11q Report of two cases and a review *Birth Defects Original Article Series* vol XII 1 5 1976
- Linarelli L G Gopalakrishna Pai K Pan S F & Rubin H M Anomalies associated with partial

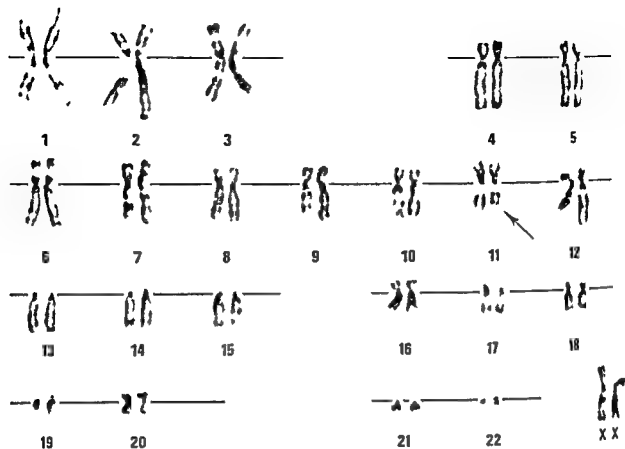


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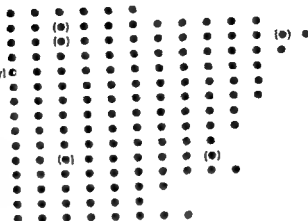


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- 3 Faust J Vogel W & Loning B A case with 46 XX del(11)(q1) *Clin Genet* 6 90 1974
- 4 Frank J & Riccardi V M The 11q syndrome *Hum Genet* 35 41 1977
- 5 Jacobsen P Hauge M Henningsen K Hobolth N Mikkelsen M & Philip J An (11 21) translocation in four generations with chromosome 11 abnormalities in the offspring *Hum Hered* 23 568 1973
- 6 Kaffe S Yau L Sachdev R Philips J & Hirschhorn K Partial deletion of long arm of chromosome 11 del(11)(q13) *Clin Genet* 12 373 1977
- 7 Larson M A Yeatman S W & Riccardi V M Deletion of 11q Report of two cases and a review *Bull Defects Original Article Series* vol XII 1 5 1976
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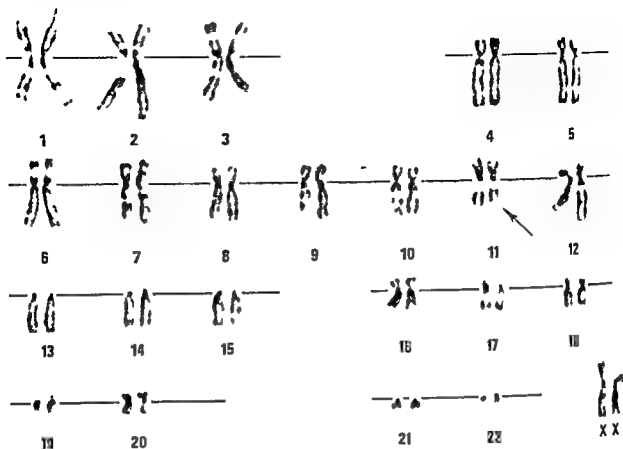


Fig 3 Trypsin Giemsa banded karyotype showing a terminal deletion (arrow) of chromosome 11 del(11)(q22)

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REVIEW OF CURRENT LITERATURE THE LITERARY JUNGLE OF CYSTIC FIBROSIS

During the last few years the literature concerning cystic fibrosis (CF) has been overwhelming. A search by Medlars for CF has yielded about twenty articles per month and added to these are review articles, proceedings from international meetings, manuals, academic theses and so on. This brief and incomplete review of the publications on CF is intended as a guide to help readers to find those works which are of most value for their special interest.

Three review articles (1-3) were published in 1976. In the *New England Journal of Medicine*, Paul di Sant Agnese & Pamela Davis (1) give a survey of the research in CF which is a direct continuation of an earlier survey in the same journal (1967). The survey is extensive and covers nearly all essential research on the pathogenesis of CF. The findings are reported in an objective way and the authors are careful not to put forward hypotheses of their own. They conclude, however, that CF must account for a generalized exocrineopathy but with morphologic and physiologic normality of the exocrine glands before the onset of the pathologic effects of disease. Control of the secretory process is a possible site of the defect. The review does not deal with clinical research on CF and gives no suggestions for the treatment and clinical care of patients with the disease.

The review by Robert E. Wood et al. (2) comprising 46 pages with 503 references covers all aspects of CF—the incidence, genetics, pathophysiology, clinical and pathologic manifestations, diagnosis, treatment, complications, psychosocial aspects and prognosis. The authors have thorough knowledge of both the research and clinical management

of CF and their description of the state of the art in CF is a result of their own experience combined with reports from the literature. The Cleveland theories regarding both the pathophysiology and treatment of CF are easily recognized. This is no drawback. The results obtained by this group both in their research and in their clinical work have been excellent and in many aspects have led the views on CF ever since the days of Leroy W. Matthews. Comprehensive and intensive therapy is mandatory in CF. The balanced discussion about must tent therapy, inhalations, antibiotic treatment, physical therapy, diet and other therapeutic aids should be read by everyone concerned with the care of CF patients.

The third review by Bowman & Barnett (3) deals mainly with those parts of CF research in which the Texas group has been most interested, namely CF factor(s) and the possibilities of prenatal diagnosis. Their review sums up the articles in the special CF issue of *Texas Reports on Biology and Medicine* (6).

The book of proceedings from the international conference Cystic Fibrosis—projections into the future (4) held in Israel in May 1976 is somewhat uneven but gives some exciting views on what can be done in CF research. Hopefulness and constructive ideas for future directions predominate even though the description of CF research given by Benke may easily give rise to despair.

There is the suggestion that cell membrane surfaces are abnormal and normal beta glucuronidase activity is abnormal and normal fatty acids are abnormal and normal serum calcium binding is abnormal and normal

- deletion of long arm of chromosome 11 *J Pediatr* 86 750 1975
- 9 Mulichy M T & Jenkyn J The 11q syndrom Another case report *Hum Genet* 36 239 1977
- 10 Turlieu C Chavin-Collin F Roubin M Thomas D & deGrouchy J Monosomie partielle 11q et trigonocephalie Un nouveau syndrome *Ann Genet* 18 257 1975
- 11 Zabel H Hansen S Hillig U & Grotig Imhof H A girl with partial long arm deletion of chromosome 11 and familial pericentric inversion of chromosome 9 *Hum Genet* 36 117 1977

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- 2 Wood R E, Boat T F & Doershuk C F Cystic fibrosis *Am Rev Resp Dis* 113 833-878 1976
- 3 Bowman B H & Barnett D R Recent advances in cystic fibrosis research *Birth defects Original article series* XII 6 197-21 1976

Proceedings etc

- 4 Mangos J A & Talamo R C (eds) *Cystic fibrosis: Projections into the future* Stratton Intercontinental Medical Book Corporation Miami Florida 1976
- 5 *Proceedings VIIIth International Cystic Fibrosis Congress Paris 1976* Imprimerie Jouve Paris 1978
- 6 Bowman B H & Barnett D R (eds) *Cystic fibrosis Texas Reports on Biology and Medicine* 34 1-719 (=whole no 1) 1976
- 7 Forstner G G (ed) *Mucus secretions and cystic fibrosis* S Karger Basel 1977
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- 11 Doershuk C F (ed) *Guide to diagnosis and management of cystic fibrosis* National Cystic Fibrosis Research Foundation Atlanta USA 1971
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polyamines in blood are high and normal RNA methylation is low and normal calcium or magnesium ATPase and sodium transport are abnormal and normal active sodium transport is affected and not affected by CF materials complement levels are elevated and normal the Timm-Horsfall glycoprotein is aggregated and normal glycosyl transferases are low high and normal the cell cycle is abnormal and normal arginine is important and not important kallikrein is abnormal and normal a CF factor can be demonstrated and not demonstrated glutathione reductase is high in red cells and low in white cells arginine esterise is abnormal and normal and CF cells have excess metachromatic material but this is normal and besides CF cells are sick and functional failures. The clinical part of the book deals with pulmonary cardiovascular gastrointestinal and nutritional features of the disease mostly focusing on a few special aspects centered mainly on the authors own investigations. It is not intended for providing clinicians with straight guidelines for treatment and they may be disappointed on this account. The second part on fundamental problems of biochemistry immunology cell and molecular biology CF factors and genetics is much more intriguing. Cells of CF affected individuals seem to be triggered into a stimulatory loop by a hitherto unknown factor. Articles by Farrell & Lundgren, Butcher and Lederberg do much to link the many contradictory findings together. The book is valuable for those who want to know where the frontier of CF research lay in 1976 and who want to get ideas about how to go on further.

The other reports of proceedings (5-8) are good complements to the Projections into the future. The proceedings of the Paris meeting (5) cover the whole field from basic to clinical research. Each of the others (6-8) is concentrated on a special problem and will therefore be of most value for those interested in the field in question. The Texas report (6) focuses on cell cultures and CF factors, the Canadian

report (7) on mucus secretions and mucociliary transport and the Belgian report (8) on chronic obstructive pulmonary disease and physical therapy.

It is not possible to make a critical review of each of the contributions in the proceedings. The books are essential for those who are interested in CF and are working on research projects in this or adjacent fields. However, there are a few points that must be underlined. CF patients should in general be more strictly defined (5-8). The diagnosis of CF is difficult and only patients with elevated sweat chlorides and at least one of the major clinical symptoms from the lungs, pancreas or meconium ileus should be included in this category. Some of the divergences might be due to improper selection of patients. Also the reference material should be well documented (5-8). To use material from patients with different kinds of inborn errors of metabolism as control material for cAMP concentrations in fibroblasts is unacceptable (7). Culture conditions (5-7) should be described in detail including the type of medium, the routine for exchange of medium, the type and concentration of serum and the number of passages. Differences in all these variables have been proven to alter the behaviour of CF cells (6).

The two manuals (9-10) give no new ideas for treatment of CF. The article by Wood et al. (2) and the last issues of the *Guides to Cystic Fibrosis* (11-12) give all the necessary information in a more balanced and objective manner. For Scandinavian readers the report on the recent symposium in Lärkrättingen (13) might also be used as a manual for the diagnosis and management.

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Review articles

- 1 di Sant Agnese P. A. & Davis P. H. Research in cystic fibrosis. *N. Engl. J. Med.* 195 481-485 534-541 and 597-602 1976

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It is not possible to make a critical review of each of the contributions in the proceedings. The books are essential for those who are interested in CF and are working on research projects in this or adjacent fields. However there are a few points that must be underlined. CF patients should in general be more strictly defined (5-6). The diagnosis of CF is difficult and only patients with elevated sweat chlorides and at least one of the major clinical symptoms from the lungs pancreas or meconium ileus should be included in this category. Some of the divergences might be due to improper selection of patients. Also the reference material should be well documented (5-8). E.g. To use material from patients with different kinds of inborn errors of metabolism as control material for cAMP concentrations in fibroblasts is unacceptable (7). Culture conditions (5-7) should be described in detail including the type of medium the routine for exchange of medium the type and concentration of serum and the number of passages. Differences in all these variables have been proven to alter the behaviour of CF cells (6).

The two manuals (9-10) give no new ideas for treatment of CF. The article by Wood et al (2) and the last issues of the Guides to Cystic Fibrosis (11-12) give all the necessary information in a more balanced and objective manner. For Scandinavian readers the report on the recent symposium in Lakartidningen (13) might also be used as a manual for the diagnosis and management.

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Review articles

- 1 di Sant Agnese P A & Davis P B Research in cystic fibrosis. *N Engl J Med* 1975; 481-485 514-541 and 597-602 1976

BOOK REVIEWS

D M Reed & F J Stanley *The epidemiology of prematurity* Urban & Schwarzenberg München 1977 370 pp illus £18.50 ISBN 3 541 71611 8

This book provides a report from a working conference held at the National Institute of Child Health and Human Development Bethesda Maryland on Nov 8-9 1976. The conference tried to answer several questions concerning the risk of prematurity among different populations i.e. the epidemiology of prematurity. With prematurity was meant various combinations of being born too early or too small or both.

Most of the 35 contributors to the conference were from the United States a few from Great Britain and one from Norway. The conference chose to go through a lot of previous work on prematurity e.g. reports from the British Perinatal Mortality Survey and from the Collaborative Perinatal Project in the U.S. It gives a good summary of factors in prematurity discussed for the last 10 to 20 years such as social and economical status race and parity.

Cigarette smoking has been much in the limelight as an important factor in low birth weight and several papers are on this subject the most interesting perhaps by Mary B. Meyer who compares cigarette smoking to living in a high altitude both conditions which deprive the fetus of oxygen. Susser and Stein give an other interesting report comprising late results from the Dutch famine study and results from nutritional supplements in pregnancy—apparently the effects of nutrition on birth weight are not simple to evaluate. Other papers deal with genetic aspects of prematurity and obstetric factors and prenatal care.

The conclusions drawn from the conference were that prematurity is a diversiform condition which we must learn more about to be able to prevent. The book is recommended to those who want a review of what is known today about the epidemiology of prematurity. It is however very few new facts or ideas to those who have followed the literature on this subject for the last ten years.

Ingrid Bjørk

C G D Brook *Practical paediatric endocrinology* 15 pp illus Academic Press London 1978 £6.30 ISBN 0-17-136050-4

According to the author's preface the aim of this book is to help paediatricians with endocrinology and endocrinologists with paediatrics. I think he has succeeded very well and presented a practical guide to the endocrine disorders of children and adolescents. The book is not a conventional text book and is primarily written for those who have some basic knowledge of paediatric endocrinology but see children with endocrine diseases only occasionally. It is however my impression that the book

could also be used by the student as an introduction to this special field of paediatrics. It is easily read and remarkably comprehensive. The first part of each chapter gives a short and usually excellent physiological review which helps the reader to understand the abnormalities and the course of action to be taken. It is possible as the author says in his preface that not all of his opinions are shared by other experts in this field but I think the advice given can always be followed without fear of any mistakes. This practical book is highly recommended but for the next edition one would wish a better proof reading the present contains too many misprints.

C G Bergstrand

J Borms & M Hebbelink (eds) *Paediatric work physiology* vol II In Medicine and Sport Series Eds E Jokl & H Hebbelink S Karger Basel 1978 178 pp illus D M 78 ISBN 3 8055 7866 3

Clinical physiological investigations are not only possible to perform in children—it is a positive surprise to anyone formerly only used to grown up patients to reveal how positive cooperative observant stimulating and kind most children are. This is perhaps most evident in small patients marked by chronic diseases like bronchial asthma or cystic fibrosis. Hopefully the results of clinical physiological investigations are rewarding to the child patient when providing better diagnoses and therapy. Certainly the work with the child patient in itself is rewarding to the physician.

This book which presents papers read at symposia at Seč Czechoslovakia in 1974 and at Bisham England in 1976 illustrates this thesis. The introduction to work physiology in children and adolescents by E Jokl starts brilliantly but ends somewhat abruptly.

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Under Clinical Pathology among other things there are found good reviews on bicycle exercise testing in cardiac patients and on the reaction to exercise in anorexia nervosa.

Under Growth and Development are assembled eight papers on physical performance as related to age maturity and physical training. The main advantage of this volume is that it gives a good idea about what can be measured in children presents different ways of doing so and provides good references for anybody interested in clinical physiology or sports medicine as applied in children. Personally I enjoyed reading it.

Mans Arborelius Jr

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A CHILD'S EXPERIENCE OF IMMINENT DEATH

INGELA NORDMARK LINDBERG and TOR LINDBERG

*From the Department of Paediatrics Malmö General Hospital
University of Lund Malmö Sweden*

ABSTRACT Nordmark Lindberg I and Lindberg T (Department of Paediatrics Malmö General Hospital University of Lund Malmö Sweden) A child's experience of imminent death Acta Paediatr Scand 68 645 1979 —We relate here the experiences and thoughts of a 7 year-old girl about her situation during the last three months of life as she lay dying of Ewing's sarcoma Our experience with this girl—and that of other children in the same situation—indicates that they have a realistic and even confident view of death Their anxiety is centred on the disease and how it affects their appearance and their activity We must often underestimate children's capacity for understanding their own situation We ought to be sensitive to appeals for contact

KEY WORDS Children and death death dying

An intensive development is taking place in paediatrics as in other spheres of natural science and we are daily inundated with data concerning physiological and pathological happenings We run an obvious risk of being sidetracked and of forgetting the child—the person—behind the disease Paediatric literature contains very little about the sick child's experiences and how it regards its own situation and its possibly imminent death More information is essential

What is written about man's thoughts feelings and experiences of his own death and that of others is excellently summarized in Feigenberg's works (5 6) Very little deals with the child and death and then mainly with the thoughts and experiences of death by healthy children (1 7 8 9 11) Based on observations interviews and psychological tests those directly concerned have interpreted the thoughts of the dying child (3 4 10 12 15) Thus the adult's evaluation unconsciously influences the reports Several works have discussed the reactions of the family and staff and have proffered advice of how the dying child should be treated (1 3 4 12) Only few works (2 13 16) describe

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It is generally accepted that children's idea of death varies with their level of development and successively reaches that of the adult (1 11) We doubt whether this is valid at least for sick children The question is whether the actual situation—the disease—and the family's reaction to it is not of more decisive importance

Here we relate a 7 year-old girl's thoughts and feelings about her imminent death and how she experienced the reaction of those around her to her situation

CASE HISTORY

Eva was a first child with a 7 year-old brother At 5 years of age she began to suffer with pains in the back She had a painful swelling over the lumbar region X ray disclosed skeletal destruction in the third lumbar vertebra The pathological anatomical diagnosis was Ewing's sarcoma She was treated at intervals with cytostatics and radiation Initially the effect was good with considerable freedom from symptoms for one year Thereafter there were signs of a slow decline with nonresponse of reflexes pains in back and legs and restricted movement The symptoms increased After two years (at age 7) she became practically confined to a wheelchair Her liver increased in size and she had pain that demanded stronger

P G Rinsley & R A Risdon *Reflux and renal scarring* British Journal of Radiology Suppl 14 1978 pp illus £3 60 (members of the British Institute of Radiology £3 00) ISBN 0306-8954

This work by Rinsley & Risdon is an important contribution to elucidate the question whether renal scarring in vesicourethral reflux (VUR) can be caused by VUR alone or if concomitant urinary infection is an obligatory requirement. In earlier, now classical studies by Hodson it was found that high pressure sterile reflux should be associated with renal scarring. Moreover, it was found that focal scar formation was related to intrarenal reflux (IRR), i.e. reflux of urine from calyx into renal parenchyma. This point has been further investigated by Rinsley & Risdon showing that susceptibility to IRR depends on the morphology of individual papillae. Cone shaped papillae were found to be resistant to IRR because of a pressure mediated closure of the slit like orifices of the collecting ducts. Compound coalescent papillae mostly found in the polar regions, a common site for focal scars, were because of their structure more easily transformed into refluxing papillae under the influence of high pressure.

In a well controlled series of experiments in the growing pig, the authors have now examined the question whether sterile IRR can cause renal scarring and the eventual role of pressure in scar formation. The results of this study are unambiguous. Under the experimental conditions used, sterile reflux even under high pressure does not cause damage to the growing kidney. Reflux in the presence of urinary infection will on the other hand readily produce renal scarring. High pressure will intensify the renal scarring by damaging and transferring non refluxing papillae into refluxing papillae.

The difference between these results and those earlier reported by Hodson is difficult to explain. One explanation may be that Hodson's animals with sterile high pressure reflux could have been subjected to transient short lived infection which certainly will be able to cause scar formation. It is reported in the present study that high pressure animals in spite of continuous chemotherapy are difficult to keep sterile because of the presence of residual urine. Another explanation may be that the two studies were performed in different species of pigs.

The applicability of these experimental findings to human VUR will have to await the results of further clinical studies. Were they directly applicable it would seem logical to reimplant ureters in children with VUR in order to prevent further renal damage. In most cases surgery could then be replaced by adequate control of infection. It is however premature to draw such conclusions. The need

for clinical follow up studies focused on the role of infection for progression of functional deterioration is obvious. The authors are very cautious in the interpretation of results and refrain from any recommendations with regard to surgery or non surgery in VUR. Their work has a new and valuable information to our understanding of reflux nephropathy. It can be highly recommended reading to all those interested in paediatric urology.

Ole Brøberg

J B Hanshaw & J A Dudgeon *Viral diseases of fetus and newborn* (vol. XXII in series Major Problems in Clinical Pediatrics) W B Saunders Co Philadelphia London and Toronto 1978 347 pp illus £14 00 1 0-7216-4500-3

Viral infections represent an important problem in perinatology. They may have short term as well as long term effects upon fetal development and upon child birth. Even if rather few viruses seem to be responsible for different kinds of congenital defects they may be important in other respects. The symptoms of disease which they may cause are extremely variable. The same agent may cause a severe or even fatal disease in fetuses or newborns while in other cases there are no obvious signs of damage in spite of a proven infection.

This little volume is a most valuable contribution to the series Major Problems in Clinical Pediatrics. The authors are well known for their important contribution to our knowledge of cytomegalic infection and congenital rubella respectively. These infections are comprehensively described in special chapters. Other chapters contain an account of herpes simplex infections, enterovirus infections, varicella zoster infections, smallpox and cinia infections with hepatitis viruses and other pathogens in the fetus and newborn. In one part of the book the pathology of placenta and cord in some viral infections is described and in another part the development of immune mechanisms in the fetus and newborn. The book also contains chapters dealing with labor, diagnosis and with prevention, treatment and anti chemotherapy.

In spite of the small size of the volume it is comprehensive and gives a lot of actual theoretical and practical information for the clinician. The book is therefore highly recommended, especially to those working in the perinatal field.

Torsten Br.

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What is written about man's thoughts, feelings and experiences of his own death and that of others is excellently summarized in Feigenberg's works (5, 6). Very little deals with the child and death, and then mainly with the thoughts and experiences of death by healthy children (1, 7, 8, 9, 11). Based on observations, interviews and psychological tests, those directly concerned have interpreted the thoughts of the dying child (3, 4, 10, 12, 15). Thus the adult's evaluation unconsciously influences the reports. Several works have discussed the reactions of the family and staff and have proffered advice of how the dying child should be treated (1, 3, 4, 12). Only few works (2, 13, 16) describe

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Here we relate a 7-year-old girl's thoughts and feelings about her imminent death and how she experienced the reaction of those around her to her situation.

CASE HISTORY

Eva was a first child with a 2-year-old brother. At 5 years of age she began to suffer with pains in the back. She had a painful swelling over the lumbar region. X-ray disclosed skeletal destruction in the third lumbar vertebra. The pathological anatomical diagnosis was Ewing's sarcoma. She was treated at intervals with cytostatics and radiation. Initially the effect was good with considerable freedom from symptoms for one year. Thereafter there were signs of a slow decline with nonresponse of reflexes, pains in back and legs and restricted movement. The symptoms increased. After two years (at age 7) she became practically confined to a wheelchair. Her liver increased in size and she had pain that demanded stronger

and stronger analgetics. At the age of 7 years and one month laminectomy and partial removal of extradural tumour tissue were carried out in unloading and pain reducing effort. After one month of relief the pain again increased, her abdomen swelled (liver enlargement and ascites) and a cough developed. She died at age 7 years and 4 months.

The family's (parents and maternal grand parents) relation with Eva was marked by an unusual frankness and lack of reserve especially the relation between Eva and her mother. Eva's illness and imminent death were realistically discussed.

In the capacity of nursery school teacher, one of us (I N L) achieved a close contact with Eva during her final three months of life. We relate here some of our conversations and experiences.

Eva's thoughts and experiences

Eva and I met for the first time a full three months before she died. She immediately made demands on me and I responded with counterdemands. This was something she was not accustomed to. For a whole week we tested each other. Eva was then transferred to the neurosurgical department for operation. On the third day after the operation, she telephoned me at home to say 'Good night'. These 'Good night' conversations continued until six days before she died. When Eva returned to the paediatric department she definitely refused all forms of physiotherapy, no attempt was made to persuade her to change her mind. She immediately noticed this and became alarmed. She was angry and grumpy. I asked her if she wished to walk down to the play therapy. This aroused loud protests. 'Why are you so silly?' you know that I can't walk. She did manage to walk as far as the door and then cried and swore. I took her into my arms, she laughed, cried and struggled a little. 'I can walk a bit but some things I can't do.' She could not explain the feeling. She said: 'One feels so lonely, nobody understands. We often spoke of the war in Vietnam and the starvation in Ethiopia. She identified with their helplessness. I am not the only one

who can't manage things, the children in Ethiopia suffer a lot of pain and get nothing to eat. But when they die they will go to some where nice. If there is much pain before dying it must be such a relief to die, otherwise one would not die. When you die you leave all those you like. But it is probably better to be dead because then you no longer suffer. But why do some people kill others?' I am going to die because I am ill and everyone is sorry but when people are killed with guns in war, there is no sorrow. When I am in heaven can I look down on you? God looks down on us now.

When Eva and I were on our way to Eva's home we passed a cemetery. Eva said: 'I will be buried here in a white coffin, all children have white coffins. Suppose my legs can't walk when I am in heaven, suppose the spider does not disappear, suppose... Someone who had visited her had said that one cannot be certain what will happen. What if I go somewhere else?'

Her way of speaking was often provoking. She wished to test the truth of what we said and asked the same question again a few days later. It was important to give positive answers. If she failed to get a positive answer she fell silent and seemed confused. Sometimes she became very irritable with those around her. 'Why do they say 'Little Eva will you have this or do that?' and try to pat me on the cheek?' I don't want to listen to their talk. They say things just because I am going to die.

Karin, 16 years of age, lay in the same ward. She had chronic and terminal myelocytic leukaemia. She had had treatment and was afraid that she would lose her hair. I have also had the same treatment that Karin is having. Eva said: 'You are given that when you have the illness we have. Does Karin know that she is going to die?' (Answer: 'We all must die some time'.) 'When you are given what Karin is having you will soon die.' (Have you mentioned that to Karin?) 'No. It would make her unhappy.' (Won't you be unhappy?) Eva did not reply. Karin does not know she is going

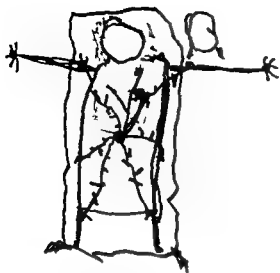


Fig. 1 Eva's sketch of her tumour. The spider's body is in the same place as the parent tumour. There are tablets on the table (to the right).

to die because she talks about what she will do when she finishes school. Eva said, 'I feel sorry for her. While we are at the shops, Kann might die. I heard the remark on the doctor's round that she has poor values.'

We noticed on our morning walks about three weeks before she died a beautiful dew spangled spider's web. When Eva saw the spider she shuddered but was fascinated by its unpleasantness. A few days later she said "It feels as though I have a spider in my back. He sits there and eats on my swelling. The swelling is so large that the spider grows bigger and bigger. He has long hairy legs. I have an itch when he moves." (Eva spoke and made a sketch at the same time. Fig. 1.) He has a large foot that fastens in certain places. Now he sits here (the lungs) which is why I am coughing. I believe that there is one in my hand too because it shakes all the time. Look how badly I draw now. Sometimes I dream that the spider wraps his legs all round me and stops my breathing. Don't tell anyone about the spider. Mammy says that when anyone has the injections I get, one has queer dreams but

I know that my spider is real I know him He squeezes my throat all the time Can t you see how large he has become? He is fat because he eats me (points to her stomach) Soon he will put his foot into my heart and then I can t live any longer Then I will join the other children

A few days later she got a little doll as large as a finger with soft arms and legs. She lay and looked at it with an expression of disgust. Look how ugly it is. You can do anything with its arms just as though it has no feelings. She bent and twisted the doll's arms and legs in all manner of positions. She probably has a spider all through her body. Eva bit off the doll's legs bit by bit. She shan't have any legs because I have none. She shan't have arms either because I have none. They shake all the time. It is that ugly spider's fault. Finally the doll's head came off. She might just as well die. You can't live without arms and legs. Throw the nasty thing away.

After that she wanted to be left in peace. She did not want to talk nor to be left by herself. Three days later she sank into coma which became increasingly deeper. Three days later she passed peacefully into the beyond.

DISCUSSION

This 7 year old girl's realistic concept of her illness and her imminent death agrees with both younger and older children's reactions, which are—in more or less detail—recorded in the literature (2, 13). These children's age related concept at death does not agree with that described concerning healthy children (1, 11).

Eva's concept of life was influenced by the priest who was her Sunday teacher. We did not feel that the thought of death held any terror for her but rather security. This she also expressed in words and sketches: she felt that God and his Angels would take care of her and that she would lie—as though in cottonwool—in their arms. On the other hand

she hated her illness and was filled with anxiety on account of it and how it affected her appearance and her mobility. A reaction also described by others (3-10-15).

We are convinced that the case of Eva is by no means isolated but she was able to say what she thought. Experience tells us that we adults rather underestimate the children's ability to understand their situation, that we must be sensitive to their appeals (cf. 14). Feigenberg (6) in his work about terminal care points to importance of one person being in close contact with the patient and becoming his confidant. This applies also to children. More over other personnel must be made aware of the phase the child is in. Eva said that some parts of the conversations should not be repeated to her relatives and to her doctor in order not to make them feel sad.

We have observed—not only through Eva—that children are sensitive to changes in the hospital routine, changes that have been made because of deteriorations in the condition, such as for instance the discontinuation of the physiotherapy for Eva. Children also react when we cease to make demands on them. Towards the end 16-year-old Karin was allowed to be careless in her room. She immediately noticed this and commented on it with some sarcasm.

We must not fail to consider as far as possible the child's view of the practical planning of the treatment. We conclude with an example. Karin was to be given special cytostatic treatment. The time for this happened to coincide with her sixteenth birthday. When told she protested: 'I don't wish to spend my last birthday in hospital'. The young ward doctor said: 'Be sensible please'. Karin who was Danish and 6 feet tall got up and looked down on the doctor. She said: 'At appellere til fornuften er verdens største slag i luften'.

(Piet Hein) (16). To appeal to the senses is the world's greatest blow in the air.

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Translated by Mr W. F. Salisbury

REFERENCES

- 1 Bellman M. Children and death. *Läkartidningen* 68: 5555 1971.
- 2 Bennholdt Thomsen C. Sterben und Tod des Kindes. *Dtsch Med Wschr* 94: 1437 1959.
- 3 Burton L. *Care of the child facing death*. Routledge & Kegan Paul, London 1974.
- 4 Easson W. M. *The dying child. The management of the child or adolescent who is dying*. C. C. Thomas Publ. Springfield Ill 1970.
- 5 Feigenberg L. Death and dying. *Läkartidningen* 68: 5811-5965 6103 1971.
- 6 Feigenberg L. *Terminal care. A method for psychological care of dying cancer patients*. Thesis. Liber Laromedel, Lund 1977.
- 7 Furman R. A. Death and the young child. *Psychanal Stud Child* 19: 321 1964.
- 8 Johansson H. & Larsson G. H. *Barns tankar om döden*. Natur och Kultur, Stockholm 1976.
- 9 Koocher H. P. Talking with children about death. *Am J Orthopsychiat* 44: 404 1974.
- 10 Morrissey J. E. A note on interviews with children facing imminent death. *Social Casework* 743 1963.
- 11 Nagy M. The child's theories concerning death. *J Genet Psychol* 73: 3 1948.
- 12 Natterson J. M. Knudson A. G. Jr. Observations concerning fear of death in fatally ill children and their mothers. *Psychosom Med* 27: 456 1960.
- 13 Roach N. *The last day of April*. California division—American Cancer Society 1974.
- 14 Solnit A. J. The dying child. *Develop Med Child Neurol* 7: 693 1965.
- 15 Spinetta J. J. Rigler D. & Karon M. Anxiety in the dying child. *Pediatrics* 57: 841 1973.
- 16 Timmons A. I. Leucemia! Is it so awful? *J Pediatr* 89: 147 1976.

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KETOTIC HYPOGLYCEMIA OF CHILDHOOD—A CLINICAL TRIAL OF SEVERAL UNIFYING ETIOLOGICAL HYPOTHESES

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ABSTRACT Dahlquist G, Gentz J, Hagenfeldt L, Larsson A, Löw H, Persson B and Zetterstrom R (Department of Paediatrics, St. Goran's Children's Hospital, the Department of Clinical Chemistry and the Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden). Ketotic hypoglycemia of childhood—a clinical trial of several unifying etiological hypotheses. *Acta Paediatr Scand* 68: 649, 1979.—We have studied 15 children referred to St. Goran's Children's Hospital because of suspected ketotic hypoglycemia. The patients were investigated according to a program designed to test several hypotheses—old and new—postulated to explain the etiology of ketotic hypoglycemia. We have used the classical ketogenic provocation with a low calorie, high fat diet and measured the blood levels of several substrates and hormones as well as the urinary excretion of certain metabolites and hormones. Out of the 15 children, 6 will fill the criteria of ketotic hypoglycemia at the time of study. The most remarkable finding in these 6 children in contrast to the other children studied was that they did not decrease their peripheral glucose utilization (measured as kg) during starvation. These 6 children seemed to be more advanced in their adaptation to ketogenic diet in all other parameters studied. The children with ketotic hypoglycemia did not differ from the other children in plasma level of cortisol or urinary excretion of nitrogen, urea, 3-methylhistidine and catecholamines. We favour the concept that the children with ketotic hypoglycemia represent the tail of the gaussian curve in the normal age-dependent development of the adaptation to starvation.

KEY WORDS Idiopathic hypoglycemia, ketotic hypoglycemia, childhood hypoglycemia

The most common form of symptomatic hypoglycemia of childhood as pointed out by McQuarrie et al. in 1954 (13) is still idiopathic. Many of these cases can be classified as the syndrome of ketotic hypoglycemia as defined by Colle & Ulstrom (7) with recurrent episodes of symptomatic hypoglycemia associated with ketonuria occurring after a period of low carbohydrate intake. Predominantly children 1.5 to 7 years old are affected and the condition remits spontaneously with growing age. The etiology of this syndrome is still unknown. The following hypotheses have been postulated: (a) a primary defect in the catecholamine response to hypoglycemia (3, 18, 20); (b) a primary defect in the muscle protein catabolism during starvation leading to a defect availability of gluconeogenic substrates

mainly in alanine (10, 14, 18); and (c) a primary defect in the cortisol response during hypoglycemia (7).

Which of the etiological hypotheses indicated above is true remains to be established—but until then the clinical management and diagnostic procedure in suspected cases of ketotic hypoglycemia has to consider several etiological principles. We have therefore studied a consecutive number of such cases admitted to our clinic. All parents had given their informed consent for the study. The children were investigated before and after provocation with a low calorie-high fat diet mainly with respect to 1) peripheral glucose utilization as measured by intravenous glucose tolerance test; 2) muscular protein catabolism as indicated by plasma alanine concentration

Table 1 Patient data

		History				Present study					
		Perinatal data									
Case No	Sex	Body weight (g) Length (cm) Gestational age (w)	SGA	Neonatal symptomatic hypoglycemia	Symptoms and signs of ketotic hypoglycemia	Age at onset (y)	Age at last attack (y)	Age (y)	Weight ^a (SD)	Length ^b (SD)	Symptomatic response to ketonic diet
Group I											
1	F	2 170 44 37	+	-	Severe vomiting unconscious 3 times	2 ⁷ / ₁₂	6 ⁴ / ₁₂	6 ³ / ₁₂	-0.5	-1	+
2	F	1 200 34 37	+	-	Convulsions only one attack	6	6	6 ³ / ₁₂	-1	-0.5	+
3	M	2 960 49 38	-	-	Convulsions only one attack	2 ¹ / ₁₂	2 ¹ / ₁₂	2 ⁹ / ₁₂	+1	+1	+
4	M	2 700 50 40	-	+	Unconscious or convulsions	2 ¹¹ / ₁₂	3 ³ / ₁₂	3 ⁹ / ₁₂	-0.7	+0.2	+
5	M	1 840 44 37	+	+	Unconscious 3 times	2 ⁹ / ₁₂	3 ⁹ / ₁₂	3 ¹⁰ / ₁₂	-0.7	-0.5	+
6	M	2 000 44 38	+	+	Convulsions 3 times	1 ⁴ / ₁₂	1 ¹¹ / ₁₂	2 ⁷ / ₁₂	-1.7	-1.8	+
Group II											
7	M	3 450 50 41	-	-	Recurrent episodes severe vomit tractable with glucose no ver hypoglyc	3	6 ⁷ / ₁₂	6 ⁹ / ₁₂	+1	+0.5	(+)
8	F	3 630 51 41	-	-	Recurrent episodes severe vomit only one low BS value	3	6 ³ / ₁₂	6 ⁷ / ₁₂	-1.7	-1.8	(+)
9	M	3 430 49 41	+	-	Convulsions only one attack	7 ⁸ / ₁₂	7 ⁴ / ₁₂	7 ⁹ / ₁₂	-1.5	-1.2	(+)
Group III											
10	M	3 500 49 43	-	-	No symptoms Dizygotic twin of No 9	-	-	7 ¹ / ₁₂	+0.8	-0.5	-
11	F	3 500 49 43	-	-	Unconscious twice (pos.) ket diet test	1 ¹ / ₁₂	6	9 ¹ / ₁₂	-0.7	-2.1	-
12	F	1 760 43 37	+	-	Unconscious twice freq attacks of morn vomit	1	3 ³ / ₁₂	9 ³ / ₁₂	-1.2	-1.0	-
13	F	2 030 44 37	+	-	No symptoms sister of No 12	-	-	12 ³ / ₁₂	-1	+0.1	-

Table 1 Patient data

		History				Present study							
Case No	Sex	Perinatal data				Symptoms and signs of ketotic hypoglycemia	Age at onset (y)	Age at last attack (y)	Present study			Symptomatic response to ketonic diet	
		Body weight (g)	Length (cm)	Gestational age (w)	SGA				Age (y)	Weight ^a (SD)	Length ^b (SD)		
14	F	3 680			-	-	Unconscious twice no ver hypoglyc	3 ¹ / ₂	4 ¹ / ₁₂	4 ¹ / ₂	+0.2	+0.2	-
		50											
		41											
15	F	7 850			-	-	Convulsions only one attack	1 ¹ / ₂	4 ¹ / ₁₂	1 ¹ / ₁₂	-0.1	+0.5	-
		49											
		40											

Small for gestational age = weight less than -2 SD in relation to gestational age (Sterky G Pediatrics 45 7 1970)

^a Swedish standards according to Karlberg et al (Acta Paediatr Scand suppl 258 1976)

+ = symptoms (i.e. vomiting, palor) and blood glucose value <2 mmol/l within 24 hours (-) = symptoms but blood glucose >2 mmol/l within 24 hours - = no symptoms blood glucose >2 mmol/l

and urinary excretion of 3 methylhistidine (21 22) and nitrogen and 3) catecholamine response as measured by urinary catecholamine excretion

MATERIALS AND METHODS

Patients

Fifteen children aged 1.5 to 17 years were studied (Table 1). Eleven of them had a history with one or more episodes of symptomatic hypoglycemia (i.e. symptoms typical for hypoglycemia associated with a blood glucose value less than 2 mmol/l and symptom relief by glucose) associated with ketonuria occurring after a short period of low energy intake. Fasting levels of blood glucose were normal between attacks. Glucagon test after 17 hours of fasting was followed by normal blood sugar rise and all children had normal leucine tolerance test. Their history and physical examination revealed no known endocrinological or metabolic disease to explain their hypoglycemia. Two children (cases 7 and 8) had recurrent episodes of severe vomiting and ketonuria and they responded favourably to intravenous glucose infusion in spite of normal blood glucose concentration. The diagnosis ketotic hypoglycemia was therefore suspected in these children. Finally two children without symptomatic hypoglycemia were included in the study: case 13 i.e. the healthy sibling of case 1 who had had frequent attacks of hypoglycemia since the neonatal period. The mother had severe toxemia during both pregnancies and at birth both girls were small for gestational age. Secondly case 10 was the healthy dizygous twin of case 9 who had two episodes of hypoglycemia after 6 years of age. They were born at term, case 10 with normal birth weight, case 9—small for gestational age (Table 1). The two asymptomatic children were

included because they might give interesting information about the influence of intrauterine growth retardation on the adaptation to starvation.

Procedure Children included in this study had been free of hypoglycemic symptoms and infectious disease for at least 7 weeks before admission to the hospital. During the first 3 days after admission the children were fed individually designed diets with the following energy content: 50% carbohydrate, 20% protein and 30% fat. The intake was carefully analysed. On the second day after admission an intravenous glucagon test was performed (30 µg of glucagon/kg body weight) and on the third day an intravenous glucose tolerance test (0.5 g/kg body weight). Venous blood samples were drawn at 8.00 a.m. on the second and third day after admission in the fasting state.

On the fourth day the children were given a ketogenic diet as described by Colle & Ulstrom (7) containing 5040 kJ per 1.73 m² body surface and day: 15% carbohydrate, 17% protein and 68% fat. The diet was provided in 3 meals at 8.00 and 12.00 a.m. and 5.00 p.m.

Venous blood samples were collected from an indwelling catheter in the femoral vein starting from 8.00 a.m. of the fourth day and subsequently every 2 hours until the test was terminated. Criteria for termination were either symptomatic hypoglycemia or one blood glucose concentration below 1.5 mmol/l or two repeated values of 2.0 mmol/l or less. The test was concluded by another intravenous glucose tolerance test. The blood samples collected in heparinized tubes were immediately centrifuged and the plasma was frozen until analysed for glucose, β-hydroxybutyrate, glycerol, alanine, insulin and cortisol. Urine was collected for 24 hours during the first and the second day after admission and furthermore during the provocation test. The urine samples were frozen until analysed for total nitrogen, urea nitrogen, catecholamine and 3-methylhistidine.

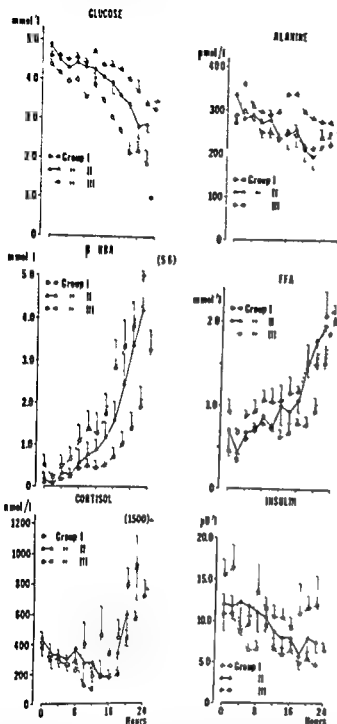


Fig. 1 Plasma concentrations of substrates and hormones during provocation with ketogenic diet in Group I (6 children who developed symptomatic hypoglycemia) Group II (3 children who developed symptoms but remained normoglycemic) and Group III (6 children who remained asymptomatic and normoglycemic). Mean \pm SE is given for each parameter.

ANALYTICAL METHODS

Plasma glucose was determined by the glucose oxidase method (GLOX AB[®] Pharmacia Uppsala Sweden). 3-hydroxybutyrate (15) and glycerol (17) were determined by enzymatic fluorometric techniques. Insulin was meas-

ured by a standard radioimmunological test system (10). Alanine was estimated by a microfluorometric modification of the method described by Williamson et al. (20). Plasma cortisol was measured by radioimmunoassay (11). Urinary catecholamine excretion was determined by a fluorescence method (1). Urea nitrogen and total nitrogen was analysed by standard techniques (kjeldahl). Urinary 1-methyl histidine and 3-methyl histidine was analysed on the short column of a Beckman[®] Unichrom amino acid analyser.

Statistical methods. Wilcoxon's nonparametric tests for both paired and not paired observations were used. When p was less than 0.05 differences were considered to be statistically significant.

RESULTS

Six children became hypoglycemic (blood glucose < 2 mmol/l) during the provocative test and 5 of them also had symptoms of hypoglycemia (see Table 1). These six children will be referred to as Group I. The mean age in this group was 4 $\frac{1}{4}$ years.

Three children (Group II) developed symptoms in the form of repeated vomiting within 24 hours during the provocative test but they remained normoglycemic and finally 6 children were asymptomatic and normoglycemic and they will be referred to as Group III. The mean ages in Group II and III were 7 and 7 $\frac{1}{4}$ years respectively.

Group III thus consisted of 3 children who had never had symptomatic hypoglycemia and 3 children with symptomatic hypoglycemia 1–6 years before the present investigation. All children in Group I had experienced an episode of symptomatic hypoglycemia within 5 months before admission.

Seven of the 11 children with a history of symptomatic hypoglycemia were born small for gestational age (weight less than -2 SD in relation to gestational age according to Swedish standards (19)). Five of the 11 patients with one or more episodes of hypoglycemia had a history of neonatal hypoglycemia (Table 1).

Metabolic response to the ketogenic provocation

Blood glucose levels in Groups I, II and III before and during the provocative test are

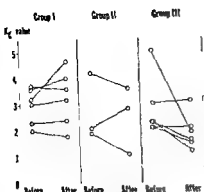


FIG. 2. Kg values before and after provocation with ketogenic diet. Group I=6 children who developed symptomatic hypoglycemia. Group II=3 children who developed symptoms but remained normoglycemic. Group III=6 children who remained asymptomatic and normoglycemic.

shown in Fig. 1. Plasma alanine during the provocation test tended to be higher in Group II than in Groups I and III (Fig. 1). Plasma concentration of FFA, glycerol and β -hydroxybutyrate tended to be higher in Group I than in Groups II and III (Fig. 1). Insulin levels tended to be lower and plasma cortisol higher in Group I compared to the Groups II and III (Fig. 1).

Glucose disappearance rate expressed as Kg values decreased significantly during the ketogenic provocation in Group III ($p < 0.025$) but not in Groups I and II, and Kg values after ketogenic provocation were significantly lower in Group III than in Group I.

Insulin response during the intravenous glucose tolerance test was measured before and immediately after the ketogenic provocation in cases 3, 4, 5 and 6 of Group I and in cases 7 and 9 in Group II and case 11 in Group III. The insulin response was similar in all patients both before and after the ketogenic provocation.

The increase in urinary excretion of epinephrine during the provocative test was similar in all groups (Table 2). Urinary norepinephrine excretion did not change significantly during the ketogenic provocative test in either group (Table 2). The urinary excretion of K_{jef}

dahl nitrogen urea and 3-methylhistidine decreased significantly during the provocation test in Groups I and III ($p < 0.03$ and $p < 0.02$ respectively). The urinary excretion of 3-methylhistidine before the ketogenic provocation was significantly lower in Group III compared to Group I ($p < 0.025$).

DISCUSSION

In the present study the most striking difference between children of Group I (i.e. with positive provocation) and Group III (i.e. with negative provocation) was that in Group I the rate of disappearance of intravenously administered glucose increased or remained unchanged during the ketogenic provocation whereas in Group III it decreased significantly. A decrease in glucose disappearance rate has been shown to occur in obese children as well as in obese women during starvation (4, 16). Our data on glucose disappearance indicate that children with ketotic hypoglycemia had a limited ability to decrease glucose oxidation during the ketogenic provocation in spite of a normal decrease in circulating insulin. Similar results were obtained by Kerr et al. (11) in a study where ^{14}C glucose was used to study glucose turnover in monozygotic twins, the smaller of whom fulfilled the diagnostic criteria for ketotic hypoglycemia. Failure to decrease glucose utilization in response to fasting or a ketogenic diet thus seems to be the main cause of hypoglycemia also in these children. In this context it is interesting to note that during starvation 20 days old intrauterine growth retarded rats were shown to be more dependent on glucose as a substrate for the brain as they had a significantly lower cerebral utilization of ketone bodies compared to normal litter mates (8).

The increase in urinary epinephrine excretion during the provocative test was similar in Groups I and II. There was thus no indication that a diminished catecholamine response contributed to the development of hypoglycemia. On the other hand the epinephrine

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REFERENCES

- Andersson B, Hovmuller S, Karlsson C G & Svensson S. Analysis of urinary catecholamines by an improved auto-analyser fluorescence method. *Reports from the Laboratory of Clinical Stress Research*, Departments of Medicine and Psychiatry, Karolinska Sjukhuset 32, 1973.
- Bier D M, Leike R D, Haymond M W, Arnold K J, Gruenke L D, Sperling M A & Kipnis D M. Measurement of true glucose production rates of infancy and childhood with 6,6-dideuteroglucose. *Diabetes* 26: 1016, 1977.
- Broberger O & Zetterstrom R. Hypoglycemia with an inability to increase the epinephrine secretion in insulin induced hypoglycemia. *J Pediatr* 56: 215, 1961.
- Cahill G, Herrera M G, Morgan A P, Soeldner J S, Steinke J, Levy P L, Reichard G A & Kipnis D M. Hormone fuel interrelationship during fasting. *J Clin Invest* 45: 1751, 1966.
- Chausin J L, Georges P, Cruzada L & Job J C. Glycemic response to 24 hour fast in normal children. III. Influence of age. *J Pediatr* 95: 711, 1977.
- Christensen N J. Hypoadrenalinemia during insulin hypoglycemia in children with ketotic hypoglycemia. *J Clin Endocrinol Metab* 38: 107, 1974.
- Colle E & Ulstrom R A. Ketotic hypoglycemia. *J Pediatr* 64: 632, 1964.
- Dahlquist G. Cerebral utilization of glucose, ketone bodies and oxygen in starving infant rats and the effect of intrauterine growth retardation. *Acta Physiol Scand* 99: 237, 1976.
- Habbrick B F, McNeish A B & Stephenson J B. P. Diagnosis of ketotic hypoglycemia of childhood. *Arch Dis Child* 46: 295, 1971.
- Haymond M W, Karl J E & Pagliara A S. Ketotic hypoglycemia: an amino acid substrate limited disorder. *J Clin Endocrinol Metab* 38: 521, 1974.
- Kerr D S, Stevens M C G, Robinson H M & Picon D J M. Hypoglycemia and the regulation of the fasting glucose metabolism in malnutrition. In L J Gardner & P Amacher (eds). *Endocrine aspects of malnutrition*. The Kroc Foundation, Santa Ynez, 1973.
- Laurell S & Tibbling G. An enzymatic fluorometric method for the determination of glycerol. *Clin Chim Acta* 13: 317, 1966.
- McQuirne I, Ulstrom R A & Ziegler M R. Random notes concerning the etiological mechanisms and treatment of spontaneous hypoglycemia. *Acta Paediatr Scand* 43: Suppl. 100, 1954.
- Pagliari A S, Karl J E, De Vivo D C, Feig R D & Kipnis D M. Hypoalaninemia: a concomitant of ketotic hypoglycemia. *J Clin Invest* 51: 1440, 1972.
- Persson B. Determination of plasma acetoacetic acid and β -hydroxy butyrate in newborn infants by an enzymatic fluorometric micromethod. *Scand J Clin Lab Invest* 29: 9, 1970.
- Persson B, Sterky G & Thorell J. Effect of low carbohydrate diet on plasma glucose, free fatty acid, glycerol, ketones and insulin during glucose tolerance tests in adolescent boys. *Metabolism* 16: 14, 1967.
- Ruder H J, Guy R L & Lipsett M B. Radioimmunoassay for cortisol in plasma and urine. *J Clin Endocrinol Metab* 35: 219, 1972.
- Sizonenko P L, Paunier L, Vallotton M B, Cuendet G Z & Marliss E H. Response to deoxy-D-glucose and to glucagon in ketotic hypoglycemia of childhood: evidence for epinephrine deficiency and altered alanine availability. *Pediatr Res* 7: 983, 1973.
- Sterky G. Swedish standard curves for intrauterine growth. *Pediatrics* 45: 7, 1970.
- Williamson P H, Lopez Vieira D & Walker B. Concentrations of free gluconeogenic amino acids in livers of rats subjected to various metabolic stresses. *Biochem J* 104: 497, 1967.
- Young V R, Alexis S D, Baliga B S & Munro H N. Metabolism of administered 3-methylhistidine. *J Biol Chem* 247: 3592, 1971.
- Young V R, Haverberg L N, Bilmazes C & Munro H N. Potential use of 3-methylhistidine excretion as an index of progressive reduction in muscle protein catabolism during starvation. *Metabolism* 22: 1429, 1973.

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DO PRE AND POSTCHALLENGE SMALL INTESTINAL BIOPSIES
HELP TO DIAGNOSE COW'S MILK PROTEIN INTOLERANCE?

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ABSTRACT Berg N O, Jakobsson I and Lindberg T (Department of Paediatrics, Malmö General Hospital and Department of Pathology, University of Lund, Sweden). Do pre- and postchallenge small intestinal biopsies help to diagnose cow's milk protein intolerance? *Acta Paediatr Scand* 68: 657-1979. Twelve infants suspected of cow's milk protein intolerance were challenged with cow's milk after at least one month of cow's milk free diet. The challenge was clinically positive in seven. Small intestinal biopsies were taken with a multipurpose capsule both pre- and postchallenge (at 24 h) in eight, prechallenge in three and postchallenge in one. Two or three biopsy specimens were taken at the same time in 15 of the 20 biopsy occasions. The morphology of the mucosa could vary from normal to slight damage at the same biopsy occasion. No difference was found in morphology judged by light microscopy between pre- and postchallenged biopsies. Light microscopy of small intestinal biopsies taken before and at 24 h after milk challenge seems of doubtful value as a routine diagnostic means in cow's milk protein intolerance.

KEY WORDS Cow's milk protein intolerance, small intestine, small intestinal biopsy.

The study of the clinical response to elimination and reintroduction of cow's milk has long been the only way to diagnose cow's milk protein intolerance (5). The small intestinal mucosa is damaged in variable degree in this disease and shows no characteristic changes (4, 10, 12, 17, 19, 21). Histological, ultrastructural and immunological changes of small intestinal mucosa after challenge with cow's milk have been reported (6, 7, 9, 11, 12, 14, 15, 17, 18, 20, 21). The value of routine light microscopy of pre- and postchallenge (at 24 h) small intestinal biopsies for the diagnosis of cow's milk protein intolerance has been discussed (3, 6, 15, 16, 17, 18, 20). The patchiness of the mucosal lesions (17, 20, 21) perhaps limits the value of this method.

To evaluate this procedure as a routine diagnostic means, we studied the morphology in multiple biopsies from twelve infants with suspected cow's milk protein intolerance. Eight were biopsied both before and after challenge with cow's milk, three before and one after. The results are reported here.

PATIENTS AND METHODS

Twelve infants were studied: seven had an heredity to allergic diseases. Symptoms (diarrhoea, vomiting, in-fantile colic and/or failure to thrive) began at age 2 weeks to 6 months. In eight, the symptoms appeared within one month on a cow's milk containing formula. Three developed symptoms on breast milk and were free of symptoms with the mothers on a cow's milk free diet (8). Lactose intolerance was excluded.

All became symptom-free and gained weight when their diet was changed to a strict cow's milk free diet (soy formula, Nutramigen® (Mead Johnson) or cow's milk free breast milk). After at least 4 weeks on this diet, they were challenged with cow's milk. After premedication with chloralhydrate, a prechallenge biopsy was taken with a hydraulic capsule (13) at the duodenojejunal flexure under fluoroscopic control.

The infants were then given increasing amounts of cow's milk (1 ml to 50 ml) every third hour and two 50 ml doses of cow's milk 17 hours and 7 hours before biopsy. A symptom-free infant had a total of 1.6 ml of cow's milk. When clinical signs appeared, the symptom giving dose was repeated once more. The postchallenge biopsy was taken from the same region as the first one 74 hours after the challenge began. Two or three specimens were usually taken a few centimeters apart.

The biopsy specimens were oriented on a millipore filter and fixed in 4% formal solution with short after fixation in Bouin's solution. They were then examined and photographed in a dissecting microscope. After embedding in paraffin or plastic, the biopsy was serially cut into 5-6 µm

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REFERENCES

1 Andersson B, Hovmuller S, Karlsson C G & Svensson B Analysis of urinary catecholamines by an improved auto-analyser fluorescence method *Reports from the Laboratory of Clinical Stress Research* Departments of Medicine and Psychiatry, Karolinska Sjukhuset 32 1973

2 Bier D M, Leake R D, Haymond M W, Arnold K J, Gruenke L D, Sperling M A & Kipnis D M Measurement of true glucose production rates of infancy and childhood with 6,6-dideuteroglucose *Diabetes* 26 1016 1977

3 Broberger O & Zetterstrom R Hypoglycemia with an inability to increase the epinephrine secretion in insulin induced hypoglycemia *J Pediatr* 56 215 1961

4 Cahill G, Herrera M G, Morgan A P, Soeldner J S, Steinke J, Levy P I, Reichard G A & Kipnis D M Hormone fuel interrelationship during fasting *J Clin Invest* 45 1751 1966

5 Chausson J L, Georges P, Calzada L & Job J C Glycemic response to 24 hour fast in normal children III Influence of age *J Pediatr* 95 711 1977

6 Christensen N J Hypoadrenalinemia during insulin hypoglycemia in children with ketotic hypoglycemia *J Clin Endocrinol Metab* 38 107 1974

7 Collie E & Ulstrom R A Ketotic hypoglycemia *J Pediatr* 64 632 1964

8 Dahlquist G Cerebral utilization of glucose, ketone bodies and oxygen in starving infant rats and the effect of intrauterine growth retardation *Acta Physiol Scand* 98 237 1976

9 Habbrick B F, McNeill A S & Stephenson J H P Diagnosis of ketotic hypoglycemia of childhood *Arch Dis Child* 46 295 1971

10 Haymond M W, Karl J E & Pagliara A S Ketotic hypoglycemia an aminoacid substrate limited disorder *J Clin Endocrinol Metab* 38 521 1974

11 Kerr D B, Stevens M C G, Robinson H M & Picon D J M Hypoglycemia and the regulation of the fasting glucose metabolism in malnutrition *In* L J Gardner & P Amacher (eds) *Endocrine aspects of malnutrition* The Kroc Foundation Santa Ynez 1973

12 Laurell S & Tibbling G An enzymatic fluorometric micromethod for the determination of glycerol *Clin Chim Acta* 13 317 1966

13 McQuarrie I, Ulstrom R A & Ziegler M R Random notes concerning the ethiological mechanisms and treatment of spontaneous hypoglycemia *Acta Paediatr Scand* 43 Suppl 100 1954

14 Pagliara A S, Karl J E, De Vivo D C, Feig R D & Kipnis D M Hypoadrenemia a concomitant of ketotic hypoglycemia *J Clin Invest* 51 1440 1972

15 Persson H Determination of plasma acetoacetate and D β hydroxy butyrate in newborn infants by an enzymatic fluorometric micromethod *Scand J Clin Lab Invest* 29 9 1970

16 Persson B, Sterky G & Thorell J Effect of low carbohydrate diet on plasma glucose free fatty acids, glycerol, ketones and insulin during glucose tolerance tests in adolescent boys *Metabolism* 16 14 1967

17 Ruder H J, Guy R L & Lipsett M B A radioimmunoassay for cortisol in plasma and urine *J Clin Endocrinol Metab* 35 219 1972

18 Sizonenko P L, Pannier L, Vallotton M B, Cuendet G Z & Marliss E B Response to deoxy D-glucose and to glucagon in ketotic hypoglycemia of childhood evidence for epinephrine deficiency and altered alanine availability *Pediatr Res* 7 993 1973

19 Sterky G Swedish standard curves for intrauterine growth *Pediatrics* 45 7 1970

20 Williamson P H, Lopez Vieira D & Walker B Concentrations of free gluconeogenic amino acids in livers of rats subjected to various metabolic stresses *Biochem J* 104 497 1967

21 Young V R, Alexis S D, Baliga B S & Munro H N Metabolism of administered 3 methylhistidine *J Biol Chem* 247 3592 1971

22 Young V R, Haverberg L N, Bilmazes C & Munro H N Potential use of 3 methylhistidine excretion as an index of progressive reduction in muscle protein catabolism during starvation *Metabolism* 22 1429 1973

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Twelve infants were studied, seven had an hereditary allergic disease. Symptoms (diarrhoea, vomiting, infantile colic and/or failure to thrive) began at age 2 weeks to 6 months. In eight the symptoms appeared within one month on a cow's milk containing formula. Three developed symptoms on breast milk and were free of symptoms with the mothers on a cow's milk free diet (8). Lactose intolerance was excluded.

All became symptomfree and gained weight when their diet was changed to a strict cow's milk free diet (soy formula Nutramigen® (Mead Johnson) or cow's milk free breast milk). After at least 4 weeks on this diet they were challenged with cow's milk. After premedication with chloralhydrate a prechallenge biopsy was taken with a hydraulic capsule (13) in the duodeno-jejunal flexure under fluoroscopic control.

The infants were then given increasing amounts of cow's milk (1 ml to 40 ml) every third hour and two 100 ml doses of cow's milk 17 hours and 2 hours before biopsy. A symptomfree infant had a total of 126 ml of cow's milk. When clinical signs appeared the symptomgiving dose was repeated once more. The postchallenge biopsy was taken from the same region as the first one 24 hours after the challenge began. Two or three specimens were usually taken a few centimeters apart.

The biopsy specimens were oriented on a millipore filter and fixed in 4% formal solution with short after fixation in Bouin's solution. They were then examined and photographed in a dissecting microscope. After embedding in paraffin or plastic the biopsy was serially cut into 5-6 µm

Table 1 Clinical and morphological results of milk challenge in cow's milk protein intolerance

Patient	Cow's milk introduced age (d)	Onset of symptoms age (d)	Cow's milk free diet age (d)	Milk challenge age (d)	Total amounts of milk (ml)	Clinical results	Morphological results			
							Prechallenge		Postchallenge	
							Villi	Histology	Villi	Histology
P O ♀	7	24	75	150	126	Positive ^a diarrhoea	(a) Leaves+ridges (b) Leaves	SL SL	(a) Leaves+ridges (b) Leaves+ridges	SL ^N
T J ♀	30	30	45	120	11	Positive vomiting diarrhoea	(a) Leaves+ridges (b) Ridges	SL SL	(a) Leaves+ridges (b) Leaves+ridges	SL SL
L Å ♀	30	60	120	150	126	Positive ^a vomiting diarrhoea	(a) Leaves (b) Leaves	SL SL	Leaves+ridges	SL
F A ♂	180	180	210	240	176	Positive diarrhoea	(a) Leaves+ridges (b) Leaves	SL SL	(a) Ridges (b) Ridges (c) Leaves+ridges	N ^a SL SL
A J ♀	2	60	120	180	126	Positive colic diarrhoea	(a) Ridges (b) Ridges (c) Ridges	N ^a N SL	- - -	- - -
C A ♀	115	115	150	235	11	Positive vomiting	(a) Ridges (b) Not evaluable	SL SL	- -	- -
O T ♂	-	30	30	90	11	Positive colic diarrhoea	-	-	(a) Finger+Leaves (b) Leaves	N ^a N ^a
M G ♂	-	14	45	90	126	Negative	Ridges	SL	Leaves+ridges	SL
A K ♂	-	45	45	180	126	Negative	Finger+leaves	N ^a	(a) Leaves (b) Leaves	SL SL
F F ♂	30	60	75	150	126	Negative	(a) Not evaluable (b) Ridges	SL SL	(a) Ridges (b) Ridges	SL SL
J P ♂	45	60	75	180	126	Negative	(a) Leaves (b) Leaves+ridges	N ^a SL	Ridges	SL
I F ♂	120	130	150	225	126	Negative	(a) Leaves (b) Leaves+ridges	N ^a SL	-	-

N=normal N^a=uncertain slight cell infiltration otherwise normal epithelial cells normal SL=slight damage in epithelial lymphocytes distinct foci of plasma cells and/or leucocytes (mostly eosinophils) slight elongation of the crypts 2-4 days after challenge

Breastfed infants with severe colic disappearing with the mother on a cow's milk free diet

sections and 1-2 µm sections respectively. The sections were stained with H&E, Eosin, PAS according to McManus and Giemsa stain for mast cells. The best oriented central cores of the specimens were used for assessment. The specimens were examined without knowledge of clinical findings.

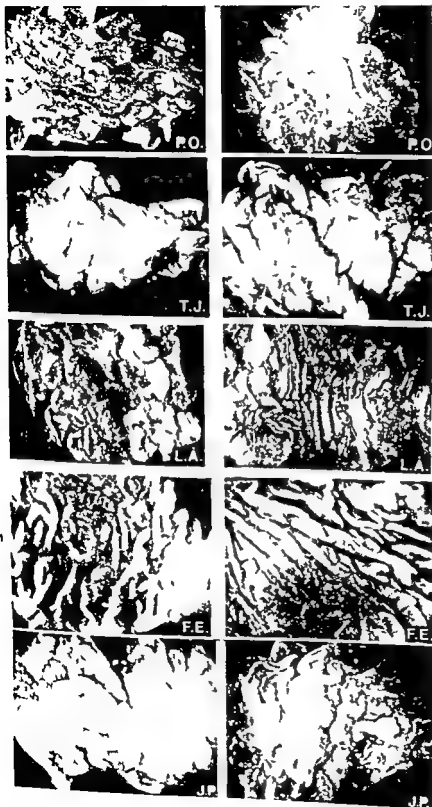
Infants with symptoms on challenge had a cow's milk free diet. Symptom-free infants continued to have a cow's milk containing formula at home and were controlled regularly. If no symptoms reappeared within twelve months the challenge was judged negative. All the infants were followed for at least one year after the challenge.

RESULTS

Table 1 gives some clinical data and the clinical and morphological results of the challenge.

The challenge was considered clinically positive in seven infants, two of whom had no symptoms until 2-4 days after the challenge. A cow's milk free formula was successfully re-introduced in all seven. They were later re-challenged with positive results but at one year of age all except two tolerated a normal

Fig. 1 Appearance of intestinal mucosa in the dissecting microscope before (to the left) and after (to the right) milk challenge in five children. For P O, T J and L Å the challenge was clinically positive but for F E and J P the challenge was negative. Slender or broader leaves and slightly increased ridging is seen for P O



diet. These two are still at two years of age on a cow's milk free diet because of eczema. The other five, with no clinical signs on challenge, continued on normal cow's milk containing diet. They had no symptoms and had gained weight normally one, two and twelve months after the challenge.

Eight infants were biopsied both before and after the challenge, three before and one after. Two or three specimens were taken at the same time at 15 out of the 20 biopsy occasions. When comparing these double specimens, ten showed the same histological picture and five showed slight variations between the specimens (Table 1). There was no difference in mucosal morphology between infants with a positive challenge and those with a negative one (Fig. 1 and Table 1). We could not observe any deterioration of the intestinal mucosa after the milk challenge compared with the prechallenged biopsies. In particular, there was no significant change in the epithelial cells, no increase in infiltrating inflammatory cells, no evident decrease in number of mast cells and no change in villous/crypt ratio.

DISCUSSION

There is a need for an objective and rapid method for diagnosing cow's milk protein intolerance. Therefore the reports (6, 7, 15, 17) on even minor (6, 7, 17) changes of morphology in intestinal biopsies 24 h after challenge with cow's milk were promising.

The present study found only slight morphological abnormalities of the intestinal mucosa. Thus in no case were distinct convolutions seen in the dissecting microscope and in well orientated sections the villous/crypt height ratio was always more than 1:1.

The study also revealed the patchy nature of the mucosal lesions (17, 20, 21). The specimens in 5 of 15 biopsy occasions showed a histological variation from normal to slight damage, irrespective of whether the biopsy was taken pre- or postchallenge and whether the challenge was clinically positive or negative.

We saw no definite changes in dissecting and light microscopy of the small intestinal mucosa at 24 hours after the challenge began compared with the prechallenge morphology, irrespective of whether the challenge was clinically positive or negative. From the infants with a positive clinical response, 13 mucosal specimens were taken on 6 pre- and 11 at 5 postchallenge biopsy occasions. On the whole, there were no more abnormalities in the postchallenge mucosal specimens. These findings are in contrast to those of Shiner *et al* (15), Sumithran & Iyengar (17) and Iyengar *et al* (6, 7). The reason for this is perhaps that these authors gave a larger challenge dose of milk and that the infants had more severe symptoms initially. In cow's milk protein intolerance, there is a varying degree in the tolerance of the mucosa to the cow's milk proteins (12) and the milk dose might be critical for the appearance of mucosal abnormalities. It is well known that the reactions can be severe after giving cow's milk to these infants (e.g. severe diarrhoea requiring intravenous fluids because of dehydration (21)) and we considered that a further increase in the amount of milk was unjustified. In our opinion, the symptoms and signs presented by the infants in this report are representative for infants with cow's milk protein intolerance in this part of the world. The infants were not treated earlier and for a longer time before challenge than in the previous studies (6, 7, 15, 17).

Finally, it is debatable whether the infants with a clinically positive challenge really had cow's milk protein intolerance and whether instead the reactions were due to some intercurrent illness. However, all reacted with similar symptoms at at least two more cow's milk challenges. Therefore we are convinced that the infants had cow's milk protein intolerance.

From this study, it can be concluded that a postchallenge biopsy at 24 h without any histological deterioration does not exclude the possibility of cow's milk protein intolerance and that the clinical result of milk challenge

does not necessarily run parallel with the morphological results judged by routine methods. This design of diagnostic procedure seems of doubtful value.

Analyses of intestinal disaccharidases or dipeptidases may be a more sensitive test of mucosal damage. Walker-Smith et al (21) found that analyses of intestinal disaccharidases in pre- and postchallenge biopsies were a helpful guide to mucosal relapse. However, these authors did the postchallenge biopsy after more than 48 h on cow's milk and the biopsies showed a significant morphological deterioration. It is known that the intestinal disaccharidase and dipeptidase activities are significantly correlated to morphological findings (2). In addition, the results of enzyme analyses can be difficult to estimate as the mucosal lesion both pre- and postchallenge can be patchy in this disorder.

We emphasize that in cow's milk protein intolerance, as in other malabsorption syndromes, a small intestinal biopsy is the only way to determine the existence of a mucosal lesion (1). Moreover, in doubtful cases, it is an invaluable means for following the effect of elimination and reintroduction of cow's milk over a longer period (11, 21).

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REFERENCES

1. Berg R, Ö Botulf S, Jakobsson I & Lindberg T. How to approach the child suspected of malabsorption. *Acta Paediatr Scand* 67: 403, 1978.
2. Berg R, Ö Dahlqvist A, Lindberg T & Norden A. Correlation between morphological alterations and enzyme activities in the mucosa of the small intestine. *Scand J Gastroenterol* 8: 703, 1973.
3. Editorial. Cow's milk protein and the small intestine. *Lancet* 1: 70, 1978.
4. Fontaine J L & Navarro J. Small intestinal biopsy in cow's milk protein allergy in infancy. *Arch Dis Child* 40: 357, 1975.
5. Goldman A S, Andersson B W, Jr, Sellers W

- A, Saperstein S, Kniker W T & Halpern S R. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 32: 425, 1963.
6. Iyngkaran M, Robinson M J, Prathap M, Sumithran E & Yadav M. Cow's milk protein sensitive enteropathy. *Arch Dis Child* 53: 70, 1978.
7. Iyngkaran N, Robinson M J, Sumithran E, Lam S M, Putehchueary S D & Yadav M. Cow's milk protein sensitive enteropathy. An important factor in prolonging diarrhoea of acute infective enteritis in early infancy. *Arch Dis Child* 53: 150, 1978.
8. Jakobsson I & Lindberg T. Cow's milk as a cause of infantile colic in breastfed infants. *Lancet* 11: 437, 1978.
9. Kilby A, Walker-Smith J A & Wood C B. Small intestinal mucosa in cow's milk allergy. *Lancet* 1: 531, 1975.
10. Kuitunen P, Visakorpi J K & Hallman N. Histopathology of duodenal mucosa in malabsorption syndrome induced by cow's milk. *Ann Paediatr* 205: 54, 1965.
11. Kuitunen P, Rapola J, Savilahti E & Visakorpi J P. Response of the jejunal mucosa to cow's milk intolerance. A light and electron microscopic study. *Acta Paediatr Scand* 67: 585, 1973.
12. Kuitunen P, Visakorpi J K, Savilahti E & Pelkonen P. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 50: 351, 1975.
13. Meuwisse H, Lindberg T & Blomberg M. A hydraulic multiple intestinal biopsy apparatus for infants. *Acta Paediatr Scand* 63: 839, 1974.
14. Shiner M, Ballard J & Smith M. The small intestinal mucosa in cow's milk allergy. *Lancet* 1: 136, 1975.
15. Shiner M, Ballard J, Brook C G D & Herman S. Intestinal biopsy in the diagnosis of cow's milk protein intolerance without acute symptoms. *Lancet* 11: 1060, 1975.
16. Shmerling D H & Rein D. Jejunal biopsy in cow's milk protein intolerance. *Lancet* 1: 77, 1978.
17. Sumithran E & Iyngkaran M. Is jejunal biopsy really necessary in cow's milk protein intolerance? *Lancet* 11: 1177, 1977.
18. Vitoria J C, Aranjuelo M & Rodriguez Sonano J. Jejunal biopsy in cow's milk protein intolerance. *Lancet* 1: 777, 1978.
19. Walker-Smith J. Cow's milk protein intolerance. Transient food intolerance of infancy. *Arch Dis Child* 50: 347, 1975.
20. Walker-Smith J A. Jejunal biopsy in cow's milk protein intolerance. *Lancet* 11: 1237, 1977.
21. Walker-Smith J A, Harrison M, Kilby B, Philips A & France N. Cow's milk sensitive enteropathy. *Arch Dis Child* 53: 375, 1978.

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REFERENCES

- Berg N O, Borulf S, Jakobsson I & Lindberg T. How to approach the child suspected of malabsorption. *Acta Paediatr Scand* 67: 403, 1978.
- Berg N O, Dahlqvist A, Lindberg T & Norden A. Correlation between morphological alterations and enzyme activities in the mucosa of the small intestine. *Scand J Gastroenterol* 8: 703, 1973.
- Editorial. Cow's milk protein and the small intestine. *Lancet* i: 707, 1978.
- Fontaine J L & Navarro J. Small intestinal biopsy in cow's milk protein allergy in infancy. *Arch Dis Child* 50: 337, 1975.
- Goldman A S, Andersson D W Jr, Sellers W A, Saperstein S, Kniker W T & Halpern S R. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 37: 475, 1963.
- Iyngkaran N, Robinson M J, Prathap M, Sumithran E & Yataw M. Cow's milk protein sensitive enteropathy. *Arch Dis Child* 53: 20, 1978.
- Iyngkaran N, Robinson M J, Sumithran E, Lam S K, Putchuchearu S D & Yadav M. Cow's milk protein sensitive enteropathy. An important factor in prolonging diarrhoea of acute infective enteritis in early infancy. *Arch Dis Child* 53: 150, 1978.
- Jakobsson I & Lindberg T. Cow's milk as a cause of infantile colic in breastfed infants. *Lancet* ii: 437, 1978.
- Kilby A, Walker Smith J A & Wood C H S. Small intestinal mucosa in cow's milk allergy. *Lancet* i: 531, 1975.
- Kuitunen P, Visakorpi J K & Hallman N. Histopathology of duodenal mucosa in malabsorption syndrome induced by cow's milk. *Ann Paediatr* 205: 54, 1965.
- Kuitunen P, Rapola J, Savilathi E & Visakorpi J P. Response of the jejunal mucosa to cow's milk intolerance. A light and electron microscopic study. *Acta Paediatr Scand* 69: 585, 1973.
- Kuitunen P, Visakorpi J K, Savilathi E & Pelkonen P. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 50: 351, 1975.
- Mouwisse G, Lindberg T & Blomberg M. A hydraulic multiple intestinal biopsy apparatus for infants. *Acta Paediatr Scand* 63: 839, 1974.
- Shiner M, Ballard J & Smith M. The small intestinal mucosa in cow's milk allergy. *Lancet* i: 136, 1975.
- Shiner M, Ballard J, Brook C G D & Herman S. Intestinal biopsy in the diagnosis of cow's milk protein intolerance without acute symptoms. *Lancet* ii: 1060, 1975.
- Shmerling D H & Rein D. Jejunal biopsy in cow's milk protein intolerance. *Lancet* i: 272, 1978.
- Sumithran E & Iyngkaran N. Is jejunal biopsy really necessary in cow's milk protein intolerance? *Lancet* ii: 1127, 1977.
- Vitoria J C, Aranjuelo M E & Rodríguez Soriano J. Jejunal biopsy in cow's milk protein intolerance. *Lancet* i: 727, 1978.
- Walker Smith J. Cow's milk protein intolerance. Transient food intolerance of infancy. *Arch Dis Child* 50: 347, 1975.
- Walker Smith J A. Jejunal biopsy in cow's milk protein intolerance. *Lancet* ii: 1737, 1977.
- Walker Smith J A, Harrison M, Kilby B, Philips A & France N. Cow's milk sensitive enteropathy. *Arch Dis Child* 53: 375, 1978.

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A PROSPECTIVE STUDY OF URINARY PROTEINS IN EARLY INFANCY

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ABSTRACT Karlsson F A HardeLL L I and Hellsing K (Departments of Clinical Research Internal Medicine Paediatrics and Clinical Chemistry University Hospital Uppsala Sweden) A prospective study of urinary proteins in early infancy *Acta Paediatr Scand* 683 1979 Urinary concentrations of protein albumin β_2 -microglobulin α amino nitrogen and creatinine were determined in forty-one full term infants on seven occasions up to six months of age Except for β_2 -microglobulin the concentrations were highest on the first day followed by a rapid decrease to a constant level within two weeks Protein diminished approximately seven fold albumin twenty fold α amino nitrogen three fold and creatinine five-fold By contrast β_2 -microglobulin a low molecular weight protein first increased three fold between day 1 and day 5 thereafter decreasing slowly 17 fold during the first three months of age The data indicate that different kidney functions mature asynchronously

KEY WORDS Albumin β_2 -microglobulin α amino nitrogen creatinine urinary concentrations kidney maturation

There is an abundance of quantitative data on protein concentrations in urines from adults and our understanding of the handling of proteins in the mature kidney is steadily growing This knowledge constitutes a necessary basis for the investigation and interpretation of pathological conditions of the kidney In addition analysis of urine may give valuable information not only concerning kidney function but also concerning metabolic and hormonal processes throughout the whole body

Surprisingly little is known about urinary proteins in early life Earlier investigations have demonstrated transitory proteinuria in newborn infants (16 20) however only qualitative measurements were performed Recently we reported urinary concentrations of albumin and β_2 -microglobulin in single urine specimens from 138 infants varying in age from full term to one year (9) Protein concentrations were highest in the group 0-1 month of age and decreased rapidly to a constant level after three months Electrophoretic analysis suggests that the proteinuria in early life is of mainly tubular origin

We now report a prospective study of 41 healthy full term infants aimed at characterizing in detail the rapid changes of urinary composition that occur in the neonatal period Further the individual acquisition of adult urinary protein pattern was examined Concentrations of total protein as well as two specific proteins albumin a high molecular weight protein and β_2 microglobulin a low molecular weight protein were determined in urines sampled on seven separate occasions during the first six months of life Also α amino nitrogen was determined as an estimate of urinary output of amino acids and creatinine values measured and evaluated as a possible indicator of diuresis

MATERIAL AND METHODS

Forty-one infants 18 boys and 23 girls born after 38 and 42 weeks of uncomplicated pregnancies were studied The deliveries were normal and all infants were in good health at birth The parents informed consent was obtained Single urine samples were collected within $\pm 10^\circ$ on the following days 1 5 15 40 90 130 and 180 Some infants did not complete the study or deliver urine on all occasions for various trivial reasons which resulted in a

Table 1 Urinary concentrations of total protein albumin β_2 -microglobulin α amino nitrogen and creatinine in single urine specimens in early infancy^a

Age (days)	n	Total protein (mg/l)	Albumin (mg/l)	β_2 microglobulin (mg/l)
1	41	494 (103-2 360)	143 (13.5-1 510)	0.278 (0.021-3.69)
5	40	176 (39.1-794)	20.8 (4.46-96.6)	1.04 (0.067-16.3)
15	29	55.3 (9.5-324)	5.59 (0.96-32.5)	0.339 (0.019-6.16)
43	28	46.4 (11.6-185)	4.35 (1.07-17.7)	0.134 (0.011-1.61)
90	25	64.4* (10.1-413)	6.31 (1.56-25.6)	0.061** (0.011-0.431)
130	23	70.0 (10.8-465)	5.78 (1.23-27.1)	0.058 (0.017-0.70)
180	19	65.7 (8.0-534)	4.55 (0.53-39.3)	0.051 (0.009-0.74)

^a Mean and range (\pm S.D.) were calculated from the logarithms of the measured values. The mean values for all compounds were tested for differences of statistical significance: data for day 1 and day 5 were compared as well as for day 5 and day 90.

* $p < 0.05$ ** $p < 0.01$ * $p < 0.001$

final yield of 71% of the expected urine samples. Analysis of a smaller group of 27 infants who completed the sample series with a 92% yield did not result in data (results not shown) that deviated from that of the entire group of 41 infants.

Urines were collected and handled as described (9). Albumin was determined by a polymer enhanced immunonephelometric method (11). β_2 -microglobulin with the I hadebras β_2 micro test (kindly supplied by Pharmacia Diagnostics, Uppsala, Sweden). Protein was quantitated by the biuret method after precipitation with phosphotungstic acid (15) using human serum albumin as reference protein. α amino nitrogen was estimated as described (10) using glycine as reference. Creatinine was determined by an Auto-Analyzer technique (Technicon N 11b).

The urine analyses were evaluated statistically after conversion to logarithmic values. Albumin and β_2 -microglobulin concentrations in infant urines when plotted on a linear scale are found to be asymmetrically distributed whereas the logarithms of the corresponding data show a more symmetric distribution suitable for statistical treatment (9). Differences in mean values were analysed with a two tailed *t* test for paired samples.

RESULTS

Table 1 presents the urinary concentrations of protein albumin β_2 -microglobulin α amino nitrogen and creatinine determined for the infants during the first six months of life. Neonatal proteinuria was regularly found at day 1: protein 494 (103-2 362) mg/l (Mean \pm S.D.) albumin 143 (13-1 511) mg/l. These high concentrations decreased rapidly: protein to 176 (39-798) mg/l albumin to 21 (4-97) mg/l on day 5. At 15 days of age a level was reached that remained constant throughout the period of the study. By con-

trast the concentrations of β_2 -microglobulin showed a different pattern—low levels at day 1, a three fold increase to maximal values at day 5: 1.04 (0.07-16.3) mg/l and thereafter a gradual 17 fold decrease over the next three months. α amino nitrogen concentrations were highest at birth: 172 (75-399) mg α amino N per liter, followed by a three fold decrease to constant values at 15 days of age. The data is displayed in Fig. 1.

The calculated ratios for protein albumin β_2 -microglobulin and α amino nitrogen to creatinine are shown in Table 2. The ratios remained constant from 15 days of age except for the β_2 -microglobulin/creatinine ratios.

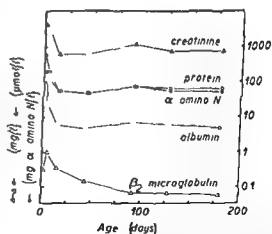


Fig. 1 Mean urinary concentration of creatinine protein α amino nitrogen albumin and β_2 -microglobulin with increasing age in 41 children studied prospectively. Creatinine (μ mol/l) α amino nitrogen (mg α amino N/l) protein albumin and β_2 -microglobulin (mg/l).

amino N α amino N(l)	Creatinine (μmol/l)
(74 5-399)	4 100 (77-71 800)
(16 4-335)	7 500 (46-13 600)
(19 6-133)	549 (101-7 970)
(16 7-125)	543 (91 6-3 750)
(7 6-187)	1 050 (199-5 550)
(15 7-117)	671 (113-3 990)
(13 3-119)	713 (63 9-7 950)

which slowly decreased and leveled off at three months of age. Note that the magnitude of the standard deviations calculated for the ratios were not less than those computed for the single values for each compound.

Protein excretion by different individuals of the same age varied considerably. Five infants had albumin concentrations of more than 600 mg/l in the first day urine, the highest being 1318 mg/l. β_2 microglobulin concentrations exceeding 4 mg/l were measured in four urines; all on day 5, the highest being 12.9 mg/l. Subsequent urinary values of albumin or β_2 microglobulin from these children were randomly distributed within the reference range. It was not possible in this prospective study based on single urine specimens to distinguish individual infants with consistently high or low protein excre-

tion patterns. Further, the amounts of urinary proteins showed no relation to the degree of weight loss and weight gain that occurred among the subjects in the neonatal period.

DISCUSSION

In studies on urines from adults, creatinine ratios can be used to normalize diuresis differences between samples when carefully timed urinary specimens are not available. However, in the early postnatal period, changes in urinary flow and creatinine concentrations are known to occur, and hence the use of creatinine as an internal indicator for the diuresis is disputable. Thus, in a study of seventeen full-term infants, creatinine levels in serum as well as urine were found to fall about 50% between day 1 and day 6 (18). During this period, the glomerular filtration rate increases two-fold (6, 13, 18) and a three- to five-fold increase in the urinary flow is observed (13, 16, 21). In the present study, a stabilization of urinary creatinine concentrations was noted two weeks after birth. However, the standard deviations computed for the ratios of the individual substances to creatinine after two weeks of age were larger than those for the substances themselves. This suggests that in the neonate/infant, other factors besides the diuresis influence creatinine output: the urinary concentrations of proteins and the amino acid

Table 2 Creatinine ratios for total protein, albumin, β_2 -microglobulin and alpha amino nitro gen measured in urines of early infancy

Age (days)	n	Total protein creatinine (mg μmol ⁻¹)	Albumin creatinine (mg μmol ⁻¹)	β_2 microglobulin creatinine (mg μmol ⁻¹)	alpha amino N creatinine (mg α amino N μmol ⁻¹)
7	11	0.118 (0.016-0.887)	0.034 (0.003-0.446)	0.067 (0.007-0.914)	0.041 (0.008-0.707)
9	40	0.070 (0.017-0.408)	0.008 (0.00-0.045)	0.443 (0.016-2.794)	0.046 (0.008-0.263)
15	79	0.101 (0.015-0.679)	0.010 (0.003-0.057)	0.617 (0.016-18.589)	0.091 (0.016-0.319)
40	11	0.085 (0.007-0.946)	0.008 (0.001-0.055)	0.746 (0.017-4.805)	0.084 (0.028-0.249)
93	5	0.059 (0.005-0.638)	0.006 (0.001-0.050)	0.053 (0.007-0.365)	0.063 (0.015-0.768)
130	23	0.117 (0.011-3.194)	0.009 (0.00-0.031)	0.091 (0.01-0.661)	0.082 (0.011-0.374)
180	19	0.105 (0.007-3.677)	0.007 (0.003-0.074)	0.067 (0.017-0.373)	0.076 (0.013-0.464)

Mean and range (\pm S.D.) were derived from the logarithms of the calculated creatinine ratios.

Table 1 Urinary concentrations of total protein albumin β -microglobulin α amino nitrogen and creatinine in single urine specimens in early infancy^a

Age (d (ys))	n	Total protein (mg/l)	Albumin (mg/l)	β_2 microglobulin (mg/l)
1	41	494 (103-2360)	143 (13.5-1510)	0.778 (0.071-3.69)
5	40	176 (39.1-798)	20.8 (4.46-96.6)	1.04** (0.067-16.3)
15	29	55.3 (9.5-324)	5.59 (0.96-32.5)	0.339 (0.019-6.16)
43	28	46.4 (11.6-185)	4.35 (1.07-17.7)	0.134 (0.011-1.61)
90	25	64.4 (10.1-413)	6.31 (1.56-25.6)	0.061* (0.011-0.331)
130	23	70.0 (10.8-465)	5.78 (1.23-27.1)	0.058 (0.012-0.770)
180	19	65.7 (8.0-519)	4.55 (0.53-39.3)	0.051 (0.009-0.87)

Mean and range (\pm SD) were calculated from the logarithms of the measured values. The mean values for compounds were tested for differences of statistical significance: data for day 1 and day 5 were compared as well for day 5 and day 90.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

final yield of 71% of the expected urine samples. Analysis of a smaller group of 27 infants who completed the sample series with a 92% yield did not result in data (results not shown) that deviated from that of the entire group of 41 infants.

Urines were collected and handled as described (9). Albumin was determined by a polymer-enhanced immunonephelometric method (11). β_2 -microglobulin with the Phadebas β_2 micro test (kindly supplied by Pharmacia Diagnostics Uppsala, Sweden). Protein was quantitated by the biuret method after precipitation with phosphotungstic acid (15) using human serum albumin as reference protein. α amino nitrogen was estimated as described (10) using glycine as reference. Creatinine was determined by an Auto-Analyzer technique (Technicon N11b).

The urine analyses were evaluated statistically after conversion to logarithmic values. Albumin and β_2 -microglobulin concentrations in infant urines when plotted on a linear scale are found to be asymmetrically distributed whereas the logarithms of the corresponding data show a more symmetrical distribution suitable for statistical treatment (9). Differences in mean values were analysed with a two-tailed *t* test for paired samples.

RESULTS

Table 1 presents the urinary concentrations of protein albumin β -microglobulin α amino nitrogen and creatinine determined for the infants during the first six months of life. Neonatal proteinuria was regularly found at day 1: protein 494 (103-2362) mg/l (Mean \pm SD), albumin 143 (13-1511) mg/l. These high concentrations decreased rapidly: protein to 176 (39-798) mg/l, albumin to 21 (4-97) mg/l on day 5. At 15 days of age a level was reached that remained constant throughout the period of the study. By con-

trast the concentrations of β -microglobulin showed a different pattern—low levels at day 1, a three fold increase to maximal values at day 5: 1.04 (0.07-16.3) mg/l and thereafter a gradual 17 fold decrease over the next three months. α amino nitrogen concentrations were highest at birth: 172 (75-399) mg α amino N per liter followed by a three fold decrease to constant values at 15 days of age. The data is displayed in Fig. 1.

The calculated ratios for protein albumin β -microglobulin and α amino nitrogen to creatinine are shown in Table 2. The ratios remained constant from 15 days of age except for the β -microglobulin/creatinine ratios.

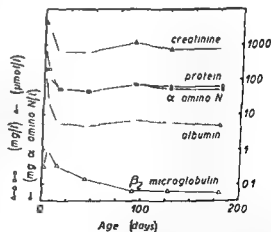


Fig. 1 Mean urinary concentration of creatinine protein α amino nitrogen albumin and β_2 -microglobulin with increasing age in 41 children studied prospectively. Creatinine (μ mol/l), α amino nitrogen (mg α amino N/l), protein, albumin and β_2 -microglobulin (mg/l).

Alfa amino N mg α amino N/l)	Creatinine (μmol/l)
77 (74.5-399)	4 100 (77-21 800)
10* (36.4-335)	7 500* (462-13 600)
11 (19.6-133)	549 (101-7 970)
15.7 (16.7-75)	545 (91.6-3 750)
0.9 (7.6-187)	1 050 (199-5 550)
4.7 (1.5-117)	671 (113-3 990)
0.1 (13.3-119)	713 (63.9-7 950)

which slowly decreased and leveled off at three months of age. Note that the magnitude of the standard deviations calculated for the ratios were not less than those computed for the single values for each compound.

Protein excretion by different individuals of the same age varied considerably. Five infants had albumin concentrations of more than 600 mg/l in the first day urine, the highest being 1318 mg/l. β_2 microglobulin concentrations exceeding 4 mg/l were measured in four urines; all on day 5, the highest being 12.9 mg/l. Subsequent urinary values of albumin or β_2 microglobulin from these children were randomly distributed within the reference range. It was not possible in this prospective study based on single urine specimens to distinguish individual infants with consistently high or low protein excre-

tion patterns. Further, the amounts of urinary proteins showed no relation to the degree of weight loss and weight gain that occurred among the subjects in the neonatal period.

DISCUSSION

In studies on urines from adults, creatinine ratios can be used to normalize diuresis differences between samples when carefully timed urinary specimens are not available. However, in the early postnatal period, changes in urinary flow and creatinine concentrations are known to occur, and hence the use of creatinine as an internal indicator for the diuresis is disputable. Thus, in a study of seventeen full-term infants, creatinine levels in serum as well as urine were found to fall about 50% between day 1 and day 6 (18). During this period, the glomerular filtration rate increases two-fold (6, 13, 18) and a three- to five-fold increase in the urinary flow is observed (13, 16, 21). In the present study, a stabilization of urinary creatinine concentrations was noted two weeks after birth. However, the standard deviations computed for the ratios of the individual substances to creatinine after two weeks of age were larger than those for the substances themselves. This suggests that in the neonate/infant, other factors besides the diuresis influence creatinine output, the urinary concentrations of proteins and the amino acid

Table 2 Creatinine ratios^a for totalprotein, albumin, β -microglobulin and alpha amino nitrogen measured in urines of early infants

Age (days)	n	Totalprotein creatinine (mg μmol ⁻¹)	Albumin creatinine (mg μmol ⁻¹)	β microglobulin creatinine (mg μmol ⁻¹)	alpha amino N creatinine (mg α amino N μmol ⁻¹)
1	41	0.118 (0.016-0.887)	0.034 (0.003-0.446)	0.067 (0.002-2.014)	0.041 (0.008-0.707)
5	40	0.070 (0.01-0.408)	0.008 (0.007-0.045)	0.443 (0.016-17.294)	0.046 (0.008-0.763)
15	79	0.101 (0.015-0.679)	0.010 (0.007-0.057)	0.617 (0.01-18.589)	0.091 (0.016-0.319)
43	8	0.081 (0.007-1.046)	0.008 (0.001-0.055)	0.746 (0.017-4.805)	0.084 (0.078-0.249)
90	5	0.059 (0.005-0.638)	0.006 (0.001-0.050)	0.053 (0.007-0.365)	0.063 (0.015-0.468)
130	3	0.117 (0.011-1.194)	0.009 (0.00-0.031)	0.091 (0.01-0.661)	0.082 (0.021-0.324)
180	19	0.105 (0.007-1.677)	0.007 (0.00-0.074)	0.067 (0.012-0.373)	0.076 (0.013-0.464)

Mean and range (\pm S.E.) were derived from the logarithms of the calculated creatinine ratios.

nitrogen. Creatinine ratios thus are of little value in estimating the absolute output of urinary compounds in the case of different single urines.

In our study the concentrations of α amino nitrogen from day 15 onwards are similar to those reported in earlier papers (3-17). No data on α amino nitrogen in immediate postnatal urine has been published to our knowledge. The high values in urines on day 1 and day 5 may reflect incomplete reabsorption of filtered amino acids. Infants are known to excrete about three times as much amino acids as children and adults (2, 3). This is generally thought to reflect a lower tubular reabsorption capacity attributable to the heterogeneity of nephrons in the neonate/infant and resulting in a relative preponderance of glomerular kidney function. The stabilization of urinary α amino nitrogen concentrations after day 15 suggests that the proximal tubules at this stage have acquired mature capacity with regard to reabsorption of this compound.

Neonatal proteinuria has been described previously in studies using semiquantitative or qualitative acid precipitation techniques. Trusch (20) detected proteinuria in 14.2% of 2837 catheterized specimens taken immediately after birth. Rhodes *et al* (16) tested 61 urines from infants less than 10 days of age and noted proteinuria in 21% of the total material. The frequency of proteinuria fell with increasing age and no full term infant over three days of age had detectable proteinuria. The rather sparse and somewhat divergent information on the subject in text books would indicate that transient proteinuria occurs during the first three days of life (1), trace amounts of protein are seldom present after the fifth day of age (7), proteinuria is frequent during the first ten days of life (12). Another book (19) refers to a study (4) which claims that the presence of urates in urine may give false positive precipitation reactions. Hence it is added that the urine of newborns is free of protein.

This study extends our earlier quantitative immunologic measurements of albumin and β microglobulin in single urine specimens in early infancy (9). Remarkably high protein concentrations were found on day 1 when approximately one third of the total protein could be accounted for by albumin. The high concentrations of total protein fell rapidly with a concomitant marked reduction in the albumin fraction reaching adult levels at day 15. At this stage qualitative tests for protein in urine from a normal infant should be negative otherwise special attention is called for.

β microglobulin, a low molecular weight protein (mol. weight 11800) which is normally reabsorbed in the proximal tubules is a useful indicator of tubular dysfunction in adults (14). The protein displayed an intriguing pattern—low levels at birth reaching a peak at day 5 and thereafter declining gradually over the next months. Serum levels of β_2 microglobulin in newborns and infants are slightly elevated compared to adults (5, 8). However, the elevation is not of such a magnitude to cause a tubular load of β -microglobulin that could account for the high urinary excretion of the protein. It is more likely that the high concentrations reflect interference with or underdevelopment of tubular capacity to reabsorb the protein. The peak at day 5 and the slow decline to the basal level at three months of age is a pattern markedly different from that of α amino nitrogen. This suggests that different functions of the proximal tubular epithelium such as amino acid reabsorption and uptake of low molecular weight proteins differentiate and mature separately.

The observations reported in this study are thought to reflect dramatic changes in the renal physiology known to occur after birth. However, the mechanisms for the proteinuria are poorly understood and the prerenal, renal and postrenal contributions to the early neonatal proteinuria is a matter of speculation. When the kidney function of a

newborn ■ evaluated it should be borne in mind that at two weeks of age proteinuria in quantitative terms should have disappeared. On the other hand at this age a high output of β_2 microglobulin and possibly other low molecular weight proteins may be present. This is a normal physiological phenomenon that will gradually decrease over the first three months of age.

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REFERENCES

- 1 Avery G B *Neonatology Pathophysiology and management of the newborn* J ■ Lippincott Comp Philadelphia 1974 ■ 493
- 2 Barlow A & McCaule R A The nitrogen partition in newborn infants urine *Arch Dis Child* 73 75 1948
- 3 Childs B Urinary excretion of free alpha amino nitrogen by normal infants and children *Proc Soc Exp Biol Med* 81 775 1955
- 4 Doxiadis S A Goldfinch M K & Cole N Proteinuria in the newborn *Lancet* II 1737 1955
- 5 Evrin P E & Wibell L The serum levels and urinary excretion of β_2 microglobulin in apparently healthy subjects *Scand J Clin Lab Invest* 29 69 1977
- 6 Guinard J ■ Torrado A Da Cunha O & Gantier E Glomerular filtration rate in the first three weeks of life *J Pediatr* 87 768 1975
- 7 Hold J L McIntosh R & Barnett H L *Pediatrics* Appel on Century-Crofts Inc New York 196 ■ 140
- 8 Somayaji L E Evrin P E & Wibell L Content of β_2 microglobulin and albumin in human amniotic fluid *Act Obstet Gynecol Scand* 5 49 1974

- 9 Karlsson F A & Hellsing K Urinary protein excretion in early infancy *J Pediatr* 89 89 1976
- 10 Khachadurian A Knox W E & Cullen A M Colorimetric ninhydrin method for total alpha amino acid of urine *J Lab Clin Med* 56 371 1960
- 11 Luzzana J & Hellsing K Polymer enhancement of automated immunological nephelometric analysis ■ illustrated by determination of urinary albumin *Clin Chem* 20 415 1974
- 12 Nelson N M Vaughan III V C & McKay R J *Textbook of pediatrics* W B Saunders Comp Philadelphia 1967 ■ 1180
- 13 Oh W Oh M A & Lind J Renal function and blood volume in newborn infant related to placental transfusion *Acta Pediatr Scand* 56 197 1966
- 14 Peterson P A Evrin P E & Berggård J Differentiation of glomerular tubular and normal proteinuria determinations of urinary excretion of β_2 microglobulin albumin and total protein *J Clin Invest* 48 1189 1969
- 15 Piscator M Proteinuria in chronic cadmium poisoning *Arch Environ Health* 5 375 1967
- 16 Rhodes P G Hammel C L & Berman L ■ Urinary constituents of the newborn infant *J Pediatr* 60 18 1962
- 17 Sereni F McNamara R Shibuya M Kretschmer N & Barnett H L Concentration in plasma and rate of urinary excretion of amino acids in premature infants *Pediatrics* 15 575 1955
- 18 Sertel H & Scopes J Rates of creatinine clearance in babies less than one week of age *Arch Dis Child* 48 717 1973
- 19 Smith C A & Nelson N M *The physiology of the newborn infant* C C Thomas Springfield 1976 p 447
- 20 Tausch M *Der Fetalharn* *Arch Gynaekol* 167 17 1936
- 21 Thompson J Observations on the urine of the newborn infant *Arch Dis Child* 19 170 1944

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BETA 2 MICROGLOBULIN AN INDICATOR OF RENAL TUBULAR MATURATION AND DYSFUNCTION IN THE NEWBORN

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ABSTRACT Aperia A & Broberger U (Department of Paediatrics, Karolinska Institutet, St. Goran's Children's Hospital and the Department of Paediatrics and Clinical Chemistry at Karolinska Hospital, Stockholm, Sweden) Beta 2-microglobulin, an indicator of renal tubular maturation and dysfunction in the newborn. *Acta Paediatr Scand* 68: 669, 1979. —The urinary excretion and proximal tubular reabsorption of beta 2-microglobulin was studied in 17 healthy newborn infants in relation to gestational and postnatal age. The effect of IRDS and non-conjugated hyperbilirubinemia on the tubular reabsorption of the protein was evaluated in 10 IRDS infants and 14 infants with non-conjugated hyperbilirubinemia. The urinary excretion of beta 2-microglobulin was determined under standardized conditions. When GFR was determined the single injection clearance method was used. The filtered load of beta 2-microglobulin was found to increase with increasing gestational age. This was due to a rise in plasma beta 2-microglobulin concentration as well as to a rise in the GFR. Although the smallest filtered load was recorded in infants with a mean GA of 32.4 weeks, these infants had a lower fractional reabsorption of the protein (88%) than infants with a mean GA of 35.0 weeks or more (98%). In infants with a GA of 35 weeks or more a glomerulo-tubular balance for beta 2-microglobulin apparently was established. In these infants the filtered load of beta 2-microglobulin increased rapidly during the first days of life. This was paralleled by an increase in the reabsorptive capacity for the protein. In infants with IRDS and in infants with non-conjugated hyperbilirubinemia the fractional reabsorption of beta 2-microglobulin was lower than in control infants of a corresponding gestational and postnatal age. This indicates that in the neonatal period the proximal tubular transporting capacity is more vulnerable than the glomerular filtration rate in states of hypoxia and hyperbilirubinemia.

KEY WORDS GFR, beta 2-microglobulin, pre-term and full term infants, idiopathic respiratory distress syndrome, hyperbilirubinemia.

In the human fetus nephrogenesis is completed at about the 36th postmenstrual week. The renal functional development is highly dependent on the renal structural development (3, 17). It can therefore be expected that in infants born before the 36th week in many aspects will differ from full term infants with regard to renal functional maturation. Arant has recently shown that the GFR is considerably lower in newborn infants with a GA less than 35 weeks (4). His results also indicate that the glomerulo-tubular balance can be different in pre term infants from that in full term infants. The fractional reabsorption of glucose and amino acids was found to be relatively lower in infants with a very low gestational age

than in full term infants. Previous studies have also shown that the excretion of sodium under basal conditions, as well as after an oral Na^+ load, is higher in pre term than in full term infants (1, 2, 25).

The renal reabsorption of Na^+ and probably also amino acids are however to a large extent dose dependent and will thus vary not only with GFR but also with serum concentration and the total balance of the respective substance. Therefore these substances are not ideal markers of renal tubular maturation.

During recent years beta 2-microglobulin

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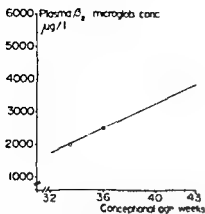


Fig 1 Plasma beta 2 microglobulin concentration in relation to conceptual age in pre-term and term infants PNA < 4 days (O) PNA > 4 days (●)

has gained interest as a marker of renal tubular function (12-24). It is a small naturally occurring protein with a molecular weight of 11800 that is easily filtered and subsequently reabsorbed in the proximal tubular cells by endocytosis and then transported to the lysosomes where the protein is degraded (19-20). There is no evidence for a transcellular transport of intact protein (5, 21). Furthermore no parts of the tubular segments distal to the proximal tubule has been shown to reabsorb any substantial amounts of the protein (18). Normally the serum concentration as well as the fractional reabsorption of beta 2 microglobulin are fairly constant (15-24).

The purpose of this study is to investigate beta 2 microglobulin as a marker of renal tubular maturation in infants born from the 32nd to the 41st gestational week. The possible deviation from normal development is evaluated in infants with idiopathic respiratory distress syndrome and in infants with non conjugated hyperbilirubinemia.

MATERIALS

This study has been performed on pre-term and term infants grouped in the following way:

1. *Healthy pre-term and term infants*: 17 infants with an uncomplicated postnatal course were divided in 3 groups with regard to the gestational age (mean \pm 1 S.D.). Group 1a: 7 infants with gestational age 32.4 ± 0.5 weeks. Group 1b: 5 infants with gestational age 35.0 ± 0.7 weeks. Group 1c: 5 infants with gestational age 40.8 ± 0.8 weeks. Infants in group 1a, b, c were studied at the postnatal

age of 4-6 days. In addition repeated studies with regard to postnatal development has been performed in all infants in group 1a at postnatal ages ranging from 1-14 days and in all infants in group 1b and c at postnatal ages 1-3 days. The total serum bilirubin did not exceed $195 \mu\text{mol/l}$ in any of these infants. All pregnancies were uneventful except for pre-term deliveries in groups 1a and b. The infants were fed breast milk in amounts of 60-100 ml/kg b.w./24 hours depending on postnatal age.

2. *Infants with idiopathic respiratory distress syndrome*: 10 infants with a mean gestational age of 34.0 weeks were studied twice: first at a mean postnatal age of 41.7 ± 16.7 hrs and then at 149.0 ± 29.0 hrs. Apgar scores were 7 or more at 1 and 5 min of age except on one case with scores of 3 and 5 respectively. The daily fluid requirement was given i.v. as 10% glucose supplemented with sodium and potassium in amounts of 60 to 170 ml/kg b.w./24 hours depending on the postnatal age. The total serum bilirubin ranged between 235 and $318 \mu\text{mol/l}$. This group of infants has been described in detail in a previous study (7).

3. *Infants with hyperbilirubinemia*: (a) 8 pre-term infants with mean gestational age 35.0 weeks were studied at a mean postnatal age of 114.1 ± 18.9 hrs. Their total serum bilirubin ranged between 255 and $316 \mu\text{mol/l}$. (b) 6 term infants with mean gestational age 39.2 weeks were studied at a mean postnatal age of 124.7 ± 7.3 hrs. The total serum bilirubin ranged between 291 and $390 \mu\text{mol/l}$.

All pregnancies were uneventful except for the pre-term deliveries. Apgar scores were 7 or more at 1 min and 8 or more at 5 min of age. All infants were fed breast milk in amounts of 60 to 120 ml/kg b.w./24 hrs depending on postnatal age.

The weight and length of all infants corresponded to the Swedish standards (11). The pre-term infants were kept in Isolette incubators. Informed parental consent was obtained in all cases studied. The protocol was approved by the Committee of Ethics at Karolinska sjukhuset.

METHODS

The urinary excretion of beta 2 microglobulin was studied during standardized fluid expansion. Breast milk diluted with water (1:1) was administered by gastric tube in an amount corresponding to 2% of the body weight during one hour and then in an amount of 0.5% every thirty minutes. Infants with IRDS were given 5.5% glucose i.v. corresponding to 1% of the body weight during the first hour. This was followed by 0.5% of the body weight per hour. When a stable diuresis was established the collection of urine started. The urine was collected after spontaneous voiding in urinary bags for a time period of 4 to 6 hours. Every portion of urine was removed and kept in the refrigerator during completion of the sampling period. The urine was then frozen and stored until analyzed. The beta 2 microglobulin excretion was calculated and expressed as the average hourly excretion per 1.73 m^2 B.S.

The beta 2 microglobulin concentration in urine and serum was determined with the Phadeba β_2 micro test (Pharmacia Diagnostics, Uppsala, Sweden).

Table 1 Urinary excretion of β_2 -microglobulin in healthy 4-6 days old pre term and term infantsValues are mean \pm S D

Gestational age weeks	37.4 \pm 0.5	35.0 \pm 0.7	40.8 \pm 0.8
Excreted β_2 -micro μ g/l 73 m B S /min	5.6 \pm 7.2	1.3 \pm 0.6	3.5 \pm 7.3
Hematocrit %	57.7 \pm 6.8	53.0 \pm 4.6	48.0 \pm 3.8
n	7	5	5

The tubular reabsorption of beta 2 microglobulin was calculated as the difference between filtered and excreted beta 2 microglobulin in urine

GFR was determined with the single injection insulin clearance method (6). For calculation of the filtered amount of beta 2 microglobulin in groups 1a-b and 3a-b previously published GFR values of comparable groups were used (7, 8).

Hematocrit was estimated in glass capillaries rinsed with heparin and centrifuged for 5 min at 12000 \times g.

The total serum bilirubin concentration was determined in duplicate with a bilirubinometer (American Optical Corp).

Conjugated bilirubin was determined with Nosselin's method (7, 8).

Gestational age was determined with the method described by Dubowitz et al. (10).

Statistical calculations included the Student's *t* test and linear regression analysis.

The following abbreviations have been used: IRDS = idiopathic respiratory distress syndrome; GA = gestational age; GFR = glomerular filtration rate; PNA = postnatal age.

RESULTS

Pre term and term control infants

The plasma concentration of beta 2 microglobulin increased with increasing conceptional age in control infants as is shown in Fig. 1. The positive correlation between plasma concentration and conceptional age is significant ($0.0025 < p < 0.005$) $r = 0.64$.

Urinary beta 2 microglobulin excretion in relation to gestational age in 4-6 days old infants. The results obtained from the studies at a postnatal age of 4-6 days in infants with a mean of GA of 32.4, 35.0 and 40.8 weeks are shown in Table 1. The urinary excretion of beta 2 microglobulin was higher ($0.0025 < p < 0.005$) in infants with a mean of GA of 32.4 weeks than in infants with a mean of 35.0 weeks who had the lowest observed urinary

excretion of beta 2 microglobulin. From a mean GA of 35.0 to 40.8 weeks the urinary excretion of beta 2 microglobulin again increased and this increase was significant ($0.05 < p < 0.025$). The urinary excretion of beta 2 microglobulin was not significantly different in infants with a mean GA of 32.4 weeks and in infants with a mean GA of 40.8 weeks.

Among the patients included in Table 1 the GFR was only determined in the group of infants with mean GA 40.8 weeks. In previous studies performed under similar conditions in this laboratory the GFR averaged 22.0 ± 6.7 ml/1.73 m² B S /min in 4-6 days old infants with a mean GA of 32.0 weeks (2) and 38.1 ± 8.1 ml/1.73 m² B S /min in 4-6 days old infants with a mean GA of 34.4 weeks (7). These values were used to calculate the average amount of filtered and reabsorbed beta 2 microglobulin in the two groups of pre term infants. In the full term infants the amount of filtered and reabsorbed beta 2 microglobulin could be calculated.

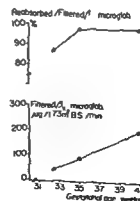


Fig. 2 Fractionally reabsorbed and filtered beta 2 microglobulin in relation to gestational age in healthy 4-6 days old pre term and term infants.

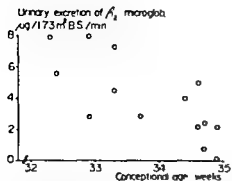


Fig. 3 Urinary excretion of beta 2 microglobulin in relation to conceptual age in pre term infants with GA < 35 weeks

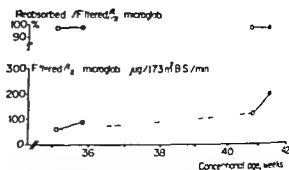


Fig. 4 Fractionally reabsorbed and filtered beta 2 microglobulin in relation to conceptual age in pre term and term infants at PNA 1-2 days (○) and 4-6 days (●)

culated in each individual patient. Changes in the amount of filtered and reabsorbed beta 2 microglobulin with increasing GA are shown in Fig. 2. There is a continuous rise in the amount of filtered beta 2 microglobulin with increasing GA. The amount of filtered beta 2 microglobulin is even when related to body surface area 5 fold higher in the infants with a GA of 40.8 weeks than in the infants with a GA of 32.4 weeks. Yet the fractional reabsorption of beta 2 microglobulin is less complete (87.2%) in the infants with a GA of 32.4 weeks than in the infants with a GA of 40.8 weeks (98.0%). Already at the gestational age of 35 weeks the fractional reabsorption of beta 2-microglobulin appears to have reached a steady level at around 98%.

Urinary beta 2 microglobulin excretion in relation to post natal age in pre term and full term control infants. The post natal development of the renal handling of beta 2 microglobulin is illustrated in Figs. 3 and 4 and Table 2. Repeated studies in infants with a

GA < 35 weeks showed that there is an indirect relationship between the urinary beta 2 microglobulin excretion and the conceptual age (see Fig. 3) when the latter ranges from 32 to 35 weeks ($r = -0.75$ ($0.0025 > p > 0.005$). In infants with a mean GA of 35.0 and 40.8 weeks the studies were performed twice at the postnatal age of 1-2 days and 4-6 days. In both the pre term and term infants there was an increase in the average excretion of beta 2 microglobulin with increasing PNA (Table 2). The increase in urinary excretion paralleled an increase in filtered load and the fractional reabsorption of beta 2 microglobulin remained constant (Fig. 4).

The relationship between filtered and reabsorbed beta 2 microglobulin. The possibility of a saturated transport capacity for beta 2 microglobulin was examined in Fig. 5. All individual values obtained from fullterm infants aged 1-5 days are included. Since there is an increase in the GFR from 1-2 days of age (34.2 ± 7.1 ml/173 m² BS/min) to 4-6 days of

Table 2 Postnatal changes of the urinary β_2 -microglobulin excretion in pre term and term healthy infants

Values are mean \pm S.D.

	GA 35 weeks		GA 40 weeks	
Post natal age hrs	28.8 \pm 2.8	136.6 \pm 22.0	37.6 \pm 11.8	103.8 \pm 11.7
Excreted β_2 micro μ g/l 173 m ² BS/min	1.0 \pm 0.9	1.3 \pm 0.6	1.5 \pm 1.3	3.5 \pm 2.3
Hematocrit %	53.6 \pm 4.0	53.0 \pm 4.6	48.8 \pm 4.8	48.0 \pm 3.8
n	5	5	5	5

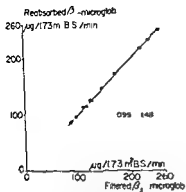


Fig 5 Relation between filtered and reabsorbed beta 2-microglobulin. Individual values in term infants with a PNA of 1-5 days (●). Solid line represents regression line for term infants. Mean values for pre-term infants (GA 35 w) at a PNA of 1-7 and 4-6 days (○).

age (53.2 ± 11.1 ml/BS/min) the filtered loads will range from $99-253 \mu\text{g/l}$ $73 \text{ m}^2 \text{BS/min}$. In the observed range of loads there is a straight line relationship between filtered and reabsorbed beta 2-microglobulin. The calculated values for the infants with a mean GA of 35 weeks aged 1-2 days and aged 4-6 days will fall on the extension of this line. Thus the increase in filtered load of beta 2-microglobulin depending on increasing GA and PNA in infants with a GA ≥ 35 weeks will not saturate the capacity to reabsorb beta 2-microglobulin.

Table 3 Filtered amount urinary excretion and reabsorption of β_2 -microglobulin in IRDS infants

	GA 34-0 weeks	
	Values are mean \pm S.D.	
Postnatal age hrs	4.7 ± 16.7	149.0 ± 9.0
Filtered β_2 -microglobulin $\mu\text{g/l}$ $73 \text{ m}^2 \text{BS/min}$	195.1 ± 59.7	113.8 ± 47.9
Urinary excretion of β_2 -microglobulin $\mu\text{g/l}$ $73 \text{ m}^2 \text{BS/min}$	9.7 ± 7.8	8.3 ± 6.7
Reabsorbed β_2 -microglobulin $\mu\text{g/l}$ $73 \text{ m}^2 \text{BS/min}$	185.3 ± 60.7	175.5 ± 43.3
Reabsorption %	94.6 ± 4.6	93.7 ± 5.1
Hematocrit %	47.3 ± 5.6	4.8 ± 6.5
n	10	10

Infants with IRDS

The plasma concentration of beta 2-microglobulin in IRDS infants ($4825 \pm 1696 \mu\text{g/l}$) was significantly higher than in pre-term controls ($2195 \pm 479 \mu\text{g/l}$) of a similar GA and PNA ($p < 0.005$). The renal handling of beta 2-microglobulin in IRDS infants is shown in Table 3. IRDS infants with a mean GA of 34.0 weeks excreted more beta 2-microglobulin in urine than control infants with a mean GA of 35.0 weeks ($0.025 > p > 0.125$). The relationship between filtered and reabsorbed beta 2-microglobulin was examined in Fig 6. Since the studies in control infants indicated a development of the reabsorptive capacity for beta 2-microglobulin until the 35th week of gestation, IRDS infants with conceptual age ≥ 35 weeks were compared to the control infants. It was found that IRDS infants consistently reabsorbed less beta 2-microglobulin than control infants at a corresponding tubular load.

Infants with hyperbilirubinemia

The plasma concentration of beta 2-microglobulin was determined in three pre-term and three term infants with non-conjugated hyperbilirubinemia. The plasma concentration was higher in pre-term hyperbilirubinemic infants ($4997 \pm 303 \mu\text{g/l}$) than in the controls with a cor-

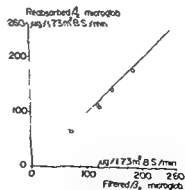


Fig 6 Relation between filtered and reabsorbed beta 2-microglobulin in IRDS infants of conceptual age ≥ 35 weeks (○). Solid line represents regression line for term infants.

Table 4 *Urinary excretion of β -microglobulin in pre term and term infants with hyperbilirubinemia*

Values are $\text{mc} \pm \text{SD}$

Gestational age weeks	35.0 ± 0.8	39.2 ± 1.2
Postnatal age hrs	114.1 ± 18.9	125.7 ± 27.3
Excreted β_2 micro		
$\mu\text{g}/1.73 \text{ m}^2 \text{ B.S./min}$	6.3 ± 4.9	6.9 ± 6.8
Hematocrit %	54.3 ± 6.8	52.5 ± 4.5
n	8	6

responding gestational age $2.195 \pm 0.978 \mu\text{g}/1$ ($p < 0.005$) but was about the same in fullterm hyperbilirubinemic infants $4.142 \pm 1.260 \mu\text{g}/1$ as in full term control infants $3.817 \pm 0.619 \mu\text{g}/1$. The urinary excretion of β_2 microglobulin (Table 4) was higher in pre term and term infants with hyperbilirubinemia than in the corresponding controls although the difference was significant only for pre term infants ($0.025 > p > 0.0125$). Individual determinations of glomerular filtration rates were obtained only in a few of the hyperbilirubinemic infants in this study. For this reason the average amount of filtered and reabsorbed β_2 microglobulin was calculated using values obtained previously for GFR in pre term hyperbilirubinemic infants ($31.1 \pm 6.3 \text{ ml}/1.73 \text{ m}^2 \text{ B.S./min}$) and full term hyperbilirubinemic infants ($36.0 \pm 7.3 \text{ ml}/1.73 \text{ m}^2 \text{ B.S./min}$) (8). The average amount of reabsorbed β_2 microglobulin when related to the filtered load of β_2 microglobulin (Fig. 7) was lower in pre term and term hyperbilirubinemic infants than in control infants.

DISCUSSION

Previous knowledge of the renal handling of β_2 microglobulin has mainly been derived from determination of β_2 microglobulin in amniotic fluid (14, 16). Apparently the amniotic content of β_2 microglobulin is determined by the fetus rather than by the mother. The concentration of β_2 microglobulin in amniotic fluid increases until the 24th week of

pregnancy. Thereafter a fall in the concentration occurs which appears to be completed at the 35th week of pregnancy. Most likely these changes are due to the combined effect of a developmental increase in production and developmental changes in the renal handling of β_2 microglobulin. This study has shown that there is a continuous rise in plasma β_2 microglobulin concentration from at least the 32nd to the 42nd conceptional week which in part must reflect an increased production of the protein with growth of the fetus and the infant. The continuous fall in the urinary β_2 microglobulin excretion that occurred from the 32nd to the 35th postmenstrual week could explain the fall in amniotic concentration of β_2 microglobulin (14, 16).

Although the infants with GA below 35 weeks had the lowest filtered load of β_2 microglobulin, the tubular reabsorption of this substance was less complete than in infants with a higher GA. This suggests rapid developmental changes of the proximal tubular cells from the 32nd to the 35th postmenstrual week. It has previously been shown (4) that the fractional excretion of glucose and of amino acids fall abruptly from the 28th to the 30th postmenstrual week and then continue to fall but more slowly until about the 36th postmenstrual week. In full term infants the glomerulo-

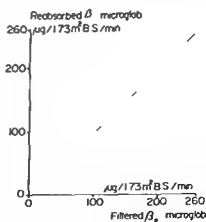


Fig. 7 Relation between average values for filtered and reabsorbed β_2 microglobulin in pre term (○) and term (●) infants with hyperbilirubinemia. Solid line represents regression line for control infants.

tubular balance for glucose is about the same as in adults (9). The fractional excretion of sodium is also relatively high in infants with gestational age below 36 weeks (2, 25). Beta 2 microglobulin, glucose and amino acids and Na⁺ are completely or to a large extent reabsorbed in the proximal tubule but with different transport pathways (20, 26, 27). It could therefore be concluded that the maturation of the renal proximal tubular cells will in most aspects be accelerated in the middle of the last trimester. This is well in agreement with previous experimental studies of the guinea pig fetus (22).

The tubular load of beta 2 microglobulin increased both with increasing gestational and postnatal age. The increase was due to a rise in plasma beta 2 microglobulin concentration as well as to a rise in glomerular filtration rate. When the filtered load increased in infants with gestational age above 35 weeks, the glomerulo-tubular balance for beta 2 microglobulin was well maintained. Thus the tubular capacity to reabsorb the protein is rapidly adapted to the demands, i.e. the filtered load in infants with gestational age above 35 weeks. In IRDS infants the filtered load of beta 2 microglobulin was higher than in control infants of corresponding GA and PNA but in the same range as in control infants of higher GA but of the same PNA. The fractional reabsorption of beta 2 microglobulin in IRDS infants was lower than in control full term infants and appeared to be lower than in control infants with conceptional age 35 weeks. The relatively low fractional reabsorption of beta 2 microglobulin in IRDS infants can then be due either to a saturation of the transport capacity for beta 2 microglobulin at the 35th-36th postmenstrual week or by an hypoxic effect on the proximal tubular epithelium. Previous studies on renal function in IRDS have shown that the GFR can be depressed as a function of hypoxia (13) but that there appears to be a threshold for this effect. Thus infants with a moderate degree of IRDS can have a normal or even a supernormal GFR (7). It should be

noted however that the fractional excretion of sodium in those infants was higher than in control infants. There are thus several indirect data that suggest that tubular function is more vulnerable than glomerular function in hypoxic infants.

In hyperbilirubinemia the fractional reabsorption of beta 2 microglobulin appeared to be lower both in pre term and in term infants than it was in control infants of corresponding gestational and postnatal age. In full term hyperbilirubinemic infants the filtered load of beta 2 microglobulin was not larger than the filtered load in the full term control infants. The relatively low fractional reabsorption of beta 2 microglobulin in the term hyperbilirubinemic infants can therefore only be attributed to an effect of hyperbilirubinemia on the proximal transport mechanism. A relatively high fractional excretion of sodium has also previously been shown in hyperbilirubinemia (8). This indicates that this condition in infants will also result in a relatively unspecific effect on the proximal tubular epithelium.

The results from this study have thus shown that glomerular functional development precedes tubular functional development until about the 34th postmenstrual week. Thereafter glomerulo-tubular balance appears to be attained at least for beta 2 microglobulin that is exclusively reabsorbed in the proximal tubule by only one transport mechanism. Even after the 34th postmenstrual week the tubular transporting capacity does however appear to be more vulnerable than the glomerular filtration rate in states of disease where the general condition of the infant is disturbed.

REFERENCES

1. Aperia A, Broberger O, Thodenius K & Zetterström R. Renal response to an oral sodium load in newborn full term infants. *Acta Paediatr Scand* 61: 670, 1972.
2. Aperia A, Broberger O, Thodenius K & Zetterström R. Developmental study of the renal response to an oral salt load in pre term infants. *Acta Paediatr Scand* 63: 517, 1974.
3. Aperia A & Larsson L. Correlation between fluid

- reabsorption and proximal tubule ultrastructure during development of the rat kidney *Acta Physiol Scand* 105 11 1979
- 4 Arant H S Developmental patterns of renal functional maturation compared in the human neonate *J Pediatr* 92 705 1978
 - 5 Bode I Ottoven P D Madsen K & Maunsbach A B Does transubular transport of protein occur in the proximal tubule? *Proc Int Symp Correlation of renal ultrastructure and function* Univ Aarhus Denmark 1978
 - 6 Broberger U Determination of glomerular filtration rate in the newborn *Acta Paediatr Scand* 67 625 1973
 - 7 Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand* 67 313 1978
 - 8 Broberger U & Aperia A Renal function in infants with hyperbilirubinemia *Acta Paediatr Scand* 68 1979
 - 9 Brodehl J Franken A & Gelissen K Maximal tubular reabsorption of glucose in infants and children *Acta Paediatr Scand* 61 413 1972
 - 10 Dubowitz L M S Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr* 77 1 1970
 - 11 Engstrom I Karlberg P Olsson T & Selstam U 1971 Copyright
 - 12 Fredriksson A Renal handling of β_2 microglobulin in experimental renal disease *Scand J Clin Lab Invest* 35 591 1975
 - 13 Guignard J P Torrado A Mazoum S M & Gautier E Renal function in respiratory distress syndrome *J Pediatr* 89 845 1976
 - 14 Hall P W & Roux J F Amniotic fluid beta 2 microglobulin concentration—an index of gestational age *Am J Obstet Gynecol* 170 56 1974
 - 15 Johansson B G & Ravnskov U The serum level and urinary excretion of a microglobulin β_2 microglobulin and lysosome in renal disease *Scand J Urol Nephrol* 6 249 1972
 - 16 Jonasson L E Evrin P E & Wibell L Content of β_2 microglobulin and albumin in human amniotic fluid *Acta Obstet Gynecol Scand* 53 49 1974
 - 17 Larsson L & Horster M Ultrastructure and fluid transport in isolated perfused developing proximal tubules *J Ultrastruct Res* 54 76 1976
 - 18 Maunsbach A B Functions of Lysosomes in Biochemistry and Pathology In J T Dingle & H B Fell (eds) *Lysosomes in biology and pathology* North Holland Publ Comp Amsterdam and London 1969 vol p 115
 - 19 Maunsbach A B Ultrastructure of the proximal tubule In S R Geiger (ed) *Handbook of physiology* Waverly Press Inc Baltimore Maryland 1979 section 8 p 31
 - 20 Maunsbach A B & Christensen E I Lysosomal accumulation and digestion of β_2 -microglobulin in kidney tubules *IRCS* 7 1737 1974
 - 21 Maunsbach A B & Christensen E I Uptake and digestion of low molecular weight protein in rat kidney tubules *Proc 6th Int Congr Nephrol* Karger Basel 1976 p 387
 - 22 Merlet Bernichou C Muffat Joly M & de Rouffignac C Renal transport of glucose and phosphate in the fetal and newborn guinea pig *Proc Int Union Physiol Sci* vol 13 Paris 1977 p 503
 - 23 Nosslin B The direct diazo reaction of bile pigment in serum *Scand J Clin Lab Invest* 17 Suppl 49 p 1 1960
 - 24 Peterson P A Evrin P E & Berggård I Differentiation of glomerular tubular and normal proteinuria *J Clin Invest* 49 1189 1969
 - 25 Siegel S M & Oh W Renal function as a marker of human fetal maturation *Acta Paediatr Scand* 65 481 1976
 - 26 Ullrich K J Renal tubular mechanism of organic solute transport *Kidney Int* 11 134 1976
 - 27 Windhager E E & Giebisch H Proximal sodium and fluid transport *Kidney Int* 9 171 1976

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THE EXCRETION OF C₆-C₁₀ DICARBOXYLIC ACIDS IN THE URINE OF NEWBORN INFANTS DURING STARVATION*Evidence for ω -oxidation of Fatty Acids in the Newborn*

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ABSTRACT Gregersen N and Ingerslev J (Research Laboratory for Metabolic Disorders, University Department of Clinical Chemistry and University Department of Obstetrics and Gynaecology, Aarhus Kommunehospital, Aarhus, Denmark). The excretion of C₆-C₁₀ dicarboxylic acids in the urine of newborn infants during starvation. *Acta Paediatr Scand* 69: 677-1979. —The excretion of C₆-C₁₀-dicarboxylic acids (i.e. adipic, suberic and sebacic acids) was measured during the three first days of life in 3 fasting newborns, 2 newborns fed with isocaloric glucose and 2 newborns given mothers' milk. On the second and third day of life the starved children excreted 27-34 mmol adipic acid/mol creatinine, 6-22 mmol suberic acid/mol creatinine and 4-7 mmol sebacic acid/mol creatinine. The excretion of C₆-C₁₀-dicarboxylic acids in the neonates given glucose or mothers' milk was for the first three days of life 0-9 mmol adipic acid/mol creatinine, 0-10 mmol suberic acid/mol creatinine and 0-4 mmol sebacic acid/mol creatinine. The latter amounts are equivalent to the excretion of dicarboxylic acids in older children. It is argued that the detected dicarboxylic acids are formed by ω -oxidation of long-chain monocarboxylic acids followed by β -oxidation and that the excreted amounts reflect ω -oxidation activity. It is speculated that the substantial ω -oxidation activity in the starving newborn serve to provide succinyl-CoA substrate for the citric acid cycle and for gluconeogenesis.

KEY WORDS Neonatal metabolism, C₆-C₁₀-dicarboxylic acids, ω -oxidation of fatty acids

Recently we reported the urinary excretion of a number of low molecular weight organic acids of the newborn infant (5). The excretion of adipic acid ranged from 0-59 mmol/mol creatinine on the first day of life and from 0-46 mmol/mol creatinine on the fourth day. The median excretion was approximately 14 mmol/mol creatinine, which is much higher than the normal adult excretion of 2-7 mmol/mol creatinine (9, 17; reference interval). Preliminary investigations in this laboratory showed that the excretion of other medium-chain dicarboxylic acids, such as suberic and sebacic acids, is also higher in neonates than in older children and adults. These dicarboxylic acids are most probably biosynthesized by ω -oxidation of long-chain monocarboxylic acids followed by β -oxidation through the ordinary fatty acid oxidation pathway (12, 13). In order

to study the possible existence of biologically significant ω -oxidation in the neonate we measured the excretion of adipic, suberic and sebacic acids in the urine of newborn infants fed with either mothers' milk, isocaloric glucose or electrolytes in water (starvation).

MATERIALS AND METHODS

Clinical material

Urine was collected from 7 healthy full-term neonates (7 females and 3 males) randomized to three groups. Each group represents a particular three days feeding program.

1. Sterile aqueous solution of 13 mmol/l potassium chloride and 5 mmol/l sodium chloride (3 neonates).

2. Sterile aqueous solution of glucose 181 g/l and electrolytes as in group 1 (7 neonates).

3. Breast milk from the hospital milk bank (2 neonates).

The solutions for groups 1 and 2 were prepared at the hospital pharmacy. All neonates were given 7 feedings per day starting 2 hours after delivery with 15-30 ml portions on day one. On days 2 and 3 the meals were increased

to 40 and 50 ml respectively. Aspiration of the stomach content was performed prior to feeding. Typically the aspirate amounted to 4–8 ml. In no instance did the 3 newborns in feeding group I reveal any clinical sign of hypoglycemia. No significant difference in mean weight loss was recorded between the groups. The neonates studied were up for adoption and were treated and observed in the prematurity ward. Informed consent was given by the head of the department.

Two reference groups were incorporated in the study: 1) 10 normal newborn at day 1–4 of life (For details of feeding program see ref. 5) 2) 10 normal children 2–9 years of age given mixed Danish diet.

Chemicals

DL 3 OH butyric acid (Na salt) was obtained from Merck AG (Darmstadt, Germany). Adipic, suberic, sebacic and diethylglutanic acids were purchased from Koch Light Laboratories Ltd (Bucks, England).

Stationary phase for gas chromatography: Dexsil 300 was obtained from Analabs Inc (North Haven, USA) and column support: chromosorb W (HP) from Koch Light Laboratories Ltd. Bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Pierce Chemical Co (Rockford, Ill, USA).

Analytical procedure

The extraction procedure was as reported previously (5) except that the extracted acids were silylated with 200 μ l of BSTFA/1% TMCS and heated at 60°C for 30 min before the analysis. The metabolic profiles were obtained by means of a Hewlett Packard 5830 gas chromatograph (5). The amounts of 3 OH butyric acid were determined from the metabolic profiles with diethylglutanic acid as internal standard. No correction for detector response factors was made. Adipic, suberic and sebacic acids were measured with diethylglutanic acid as internal standard, mass fragmentographically by an AEI (KRATOS) MS 30 mass spectrometer supplemented with a 6-channel selected ion detector (SID) unit. For all the compounds including the internal standard, M 15 was used as the ion to be monitored. The standard curves were linear from 0–55 mmol/mol creatinine and the within-day imprecision was <10% for the three dicarboxylic acids. Details of the analysis will be reported elsewhere.

RESULTS

A gas chromatogram of a urinary metabolic profile of organic acids from a starved neonate is demonstrated in Fig. 1. The profiles of the investigated children were recorded in order to detect any gross difference in the excretion pattern in the three feeding groups. The only significant difference was the excretion of 3 OH butyric acid and the dicarboxylic acids.

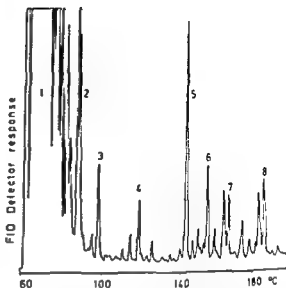


Fig. 1 Flame ionisation detector (FID) response of a silylated metabolic profile of organic acids from a starved infant the second day of life. The peaks represent: 1) solvent; 2) lactic acid; 3) 3 OH butyric acid; 4) succinic acid; 5) adipic acid; 6) diethylglutanic acid (internal standard); 7) suberic acid; and 8) sebacic acid + hippuric acid (gas chromatographic conditions see ref. (5)).

The concentration of 3 OH butyric acid was determined directly from the profiles while the dicarboxylic acids were quantitated by means of the more sensitive and specific mass fragmentographic method. A mass fragmentographic profile of the dicarboxylic acids is shown in Fig. 2. The data obtained are listed in Table 1. The numbers are too few for statistical evaluation. In the present study the excretion pattern of the infants fed with mothers milk or glucose are comparable to that of older children (Table 1). From the second day on the excretion of adipic acid and suberic acid is clearly much higher in the starved than in the fed infants.

DISCUSSION

During the first days of life until breast feeding is sufficiently instituted the normal newborn infant is in a state of starvation and depends almost entirely on endogenous fat for energy requirements (8–14). In the present study we investigated the particular metabolic situation of absolute starvation in the neonatal

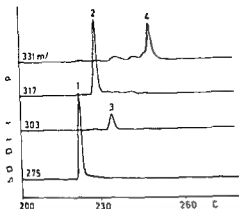


Fig. 2 Selected ion detection (SID) responses of the profiles of ion fragments m/e 775, 303 and 331 in the silylated extract from the same urine as that in Fig. 1. The peaks represent 1) adipic acid, 2) diethylglutanic acid (internal standard), 3) suberic acid and 4) sebacic acid. The gas chromatographic column was a 180 cm \times 3 mm (i.d.) glass coil packed with 3% Dexsil 300 on Chromosorb W (HP). The column temperature was programmed from 200°C at 15°C/min to 300°C. The helium carrier flowrate was 30 ml/min. The injection port and the glass sample line including the jet separator between the gas chromatograph and the mass spectrometer were 250°C and the ion source temperature was 175°C. Ionizing and accelerating potentials were 70 eV and 4 kV, respectively.

period in one group of infants while we prevented the starvation in two other groups fed with either sufficient amounts of glucose or mothers milk to cope with energy demands. The excretion of 3-OH butyric acid increases to moderate levels in the fasting groups serve to characterise the state of excessive lipid catabolism. This metabolic situation is also reflected in the urinary excretion of substantial amounts of C_6 – C_{10} dicarboxylic acids in the starved infants compared to the low excretion of these acids in the fed newborns.

In light of the investigations of Pettersen (10, 11, 12, 13) and Bjorkhem (1, 2) it is most probable that the source of urinary dicarboxylic acids in starvation are fatty acids ω -oxidised to long-chain dicarboxylic acids followed by β -oxidation. The increased ω -oxidation in liver homogenates from starved and diabetic rats (1) indicates a positive correlation between the amounts of dicarboxylic acids ex-

creted and ω oxidation in the intact organism. Therefore a substantial excretion of dicarboxylic acids in the starved group most probably reflects the degree of ω -oxidation. On the other hand in the groups fed with carbohydrate or milk the ω -oxidation was comparable to that found in older non fasting children. The finding of increased ω -oxidation in starved infants is in accordance with Bjorkhem who demonstrated an inverse relationship between ω -oxidation and glyceride biosynthesis (2) which is low during starvation (7). The relative metabolic importance of ω oxidation compared to the ordinary β oxidation cannot be determined from the presented results as it is probable that the excreted amounts of dicarboxylic acids represents an overflow of ω oxidised fatty acids. The present results however and those of Bjorkhem (1, 2) and Pettersen (10, 11) very strongly indicate that ω -oxidation is more important in states of excessive fat catabolism i.e. starvation and diabetes than in the normal state.

Wada & Usami (16) estimated that about 15% of palmitic acid was subjected to ω oxidation followed by β -oxidation in starved diabetic rats. Hemmelgarn and coworkers estimate an initial ω -oxidation percentage of 40 in non ketotic diabetic rats whereas the remaining fatty acid decomposition is due to primary β -oxidation (4). These figures suggest that ω oxidation plays a significant biological role. The excreted dicarboxylic acids must have been derived from their CoA-derivatives. If adipyl CoA is β -oxidised succinyl CoA will be formed. Succinyl CoA may serve as substrate for the citric acid cycle and consequently as a gluconeogenic precursor. The finding of Wada & Usami (16) corroborate this hypothesis. After administration of dicarboxylic acids to starved rats a decrease in blood ketone bodies was detected and a higher incorporation of ^{14}C into blood glucose from 1- ^{14}C -dicarboxylic acids than from 1- ^{14}C monocarboxylic acids was recorded. In the newborn the biological consequence of ω -oxidation of fatty acids resulting in the formation

to 40 and 50 ml respectively. Aspiration of the stomach content was performed prior to feeding. Typically the aspirate amounted to 4–8 ml. In no instance did the 3 newborns in feeding group 1 reveal any clinical sign of hypoglycemia. No significant difference in mean weight loss was recorded between the groups. The neonates studied were up for adoption and were treated and observed in the prematurity ward. Informed consent was given by the head of the department.

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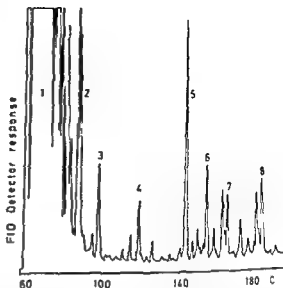


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DISCUSSION

During the first days of life until breast feeding is sufficiently instituted the normal newborn infant is in a state of starvation and depends almost entirely on endogenous fat for energy requirements (8–14). In the present study we investigated the particular metabolic situation of absolute starvation in the neonatal

- 12 Pettersen J E Formation of *n* hexanedioic acid from hexadecanoic acid by an initial ω -oxidation in ketotic rats *Clin Chim Acta* 41 731 1972
- 13 Pettersen J E In vitro studies on the metabolism of hexadecanedioic acid and its mono L-carnitine ester *Biochim Biophys Acta* 306 1 1973
- 14 Shelley H J Carbohydrate reserves in the newborn infant *Br Med J* 1 273 1964
- 15 Williams L Kaye R & Kumagai M Studies on the mechanism of ketosis in infants and children *Am J Dis Child* 94 499 1957
- 16 Wada F & Usami M Studies on fatty acid ω -oxida-

tion antiketogenic effect and glyconeogenicity of dicarboxylic acids *Biochim Biophys Acta* 487 261 1977

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Table 1 The urinary excretion of 3 OH butyric acid, adipic acid, suberic acid and sebacic acid in starved, glucose fed and milk fed newborn infants (ranges in mmol/mol creatinine)

Day of life	3 OH butyric acid	Adipic acid	Suberic acid	Sebacic acid
Starved group				
1	ND ^a -14 (2)*	7-19 (2)	7-10 (2)	2-4 (*)
2	38-111 (3)	27-65 (3)	8-19 (3)	4-6 (3)
3	50-353 (3)	40-84 (3)	6-22 (3)	4-7 (3)
Glucose fed group				
1	ND-11 (2)	1-4 (2)	2-4 (2)	nd-2 ^a (*)
2	10-26 (2)	nd-5 (2)	1-5 (2)	nd-2 (*)
3	15-17 (*)	2-8 (2)	nd-6 (2)	nd-3 (2)
Mother's milk group				
1	12-15 (2)	8-9 (2)	3-10 (2)	1-4 (2)
2	10-12 (*)	8-9 (2)	4-8 (2)	2-4 (*)
3	29-30 (2)	4-7 (2)	3-4 (*)	2-3 (*)
Normal newborn infants				
1-4	ND-39 (18) ND ^a	2-18 (10) 12 ^a	2-12 (10) 4 ^a	1-7 (10) 3 ^a
Normal older children				
2-9 years	14-32 (5) 17 ^a	2-12 (10) 4 ^a	1-6 (10) 1 ^a	1-4 (10) 2 ^a

The figures in parentheses represent the number of children investigated

* ND not detectable limit of detection approx. 10 mmol/mol creat

nd not detectable limit of detection approx. 1 mmol/mol creat

^a Median values

of succinyl CoA may be a citric acid cycle activity independent of amino acid and carbohydrate metabolism. This may explain why some newborns with very low blood glucose do not show clinical signs of hypoglycemia (3, 9) and it might be speculated that this metabolic influence of ω oxidation is responsible for the remarkable neonatal resistance to ketosis (6, 15).

ACKNOWLEDGEMENT

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REFERENCES

- 1 Björkhem I. ω -oxidation of stearic acid in the normal starved and diabetic rat liver. *Eur J Biochem* 40: 415 1973.
- 2 Björkhem I. On the mechanism of regulation of ω oxidation of fatty acids. *J Biol Chem* 251: 5259 1976.
- 3 Cornblath M, Baens G S & Lundeen E. Hypoglycemia in premature infants. *Am J Dis Child* 107: 729 1961.
- 4 Hemmelgarn B, Kumaran K & Landau B R. Role of ω -oxidation of fatty acids in formation of the acetyl unit for acetylation. *J Biol Chem* 252: 4319 1977.
- 5 Gregersen N, Ingerslev J & Rasmussen K. Low molecular weight organic acids in the urine of the newborn. *Acta Paediatr Scand* 66: 85 1977.
- 6 Heymann W. Metabolism studies on age disposition to ketosis in human beings. *J Pediatr* 12: 21 1974.
- 7 Mayes P A & Felts J M. Regulation of fat metabolism in the liver. *Nature* 215: 716 1967.
- 8 McCance K A & Strangeways W M. Protein catabolism and oxygen consumption during starvation in infants, young adults and old men. *Br J Nutr* 8: 21 1954.
- 9 Pagliara A S, Karl I E, Haymond M & Lipins D M. Hypoglycemia in infancy and childhood. Part I. *J Pediatr* 87: 365 1973.
- 10 Pettersen J E, Jellum E & Eldjarn L. The occurrence of adipic and suberic acids in the urine from ketotic patients. *Chin Chim Acta* 38: 17 1977.
- 11 Pettersen J E. Urinary excretion of *n*-hexanedioic and *n*-octanedioic acid in juvenile diabetics with ketonuria. *Diabetes* 23: 16 1974.

HYPERLIPOPROTEINEMIA IN NEWBORN INFANTS

A Study of 1025 Families

GUNNAR E. ANDERSEN, PER LOUS and BENT FRIIS HANSEN

From the Neonatal Department, Rigshospitalet and the Department of Clinical Chemistry, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark

ABSTRACT Andersen G E, Lous P and Friis Hansen B (Neonatal Department, Rigshospitalet and the Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark). Hyperlipoproteinemia in newborn infants. A study of 1025 families. *Acta Paediatr Scand* 68: 683, 1979. —As part of a screening study for the detection of hyperlipoproteinemia in 10 000 newborns, cord serum lipids and lipoproteins were measured in detail in 1025 infants. Elevated cord serum VLDL/LDL-cholesterol could easily be identified by a rapid turbidimetric estimation of cord serum VLDL/LDL. Cord serum VLDL/LDL-cholesterol was found to be significantly higher than normal in premature, asphyxiated and beta-methasone/phenobarbital/ritodrine-treated infants. Other obstetric complications, however, were not associated with hyperlipoproteinemia. Furthermore, all 2040 parents had their serum cholesterol determined. 3 parents had familial hypercholesterolemia (FH). One child also had FH, though her cord serum total cholesterol and VLDL/LDL-cholesterol were normal. The 2 other children of the 3 FH parents had normal lipids and lipoproteins both at birth and follow-up.

KEY WORDS Cholesterol, triglyceride, lipoproteins, newborns.

Within the last few years several studies have been published which suggest that elevated lipids and lipoproteins in newborns may reflect inherited hyperlipoproteinemia (1-5), prematurity (6), postmaturity (7), IUGR (6, 8), diabetes in the mother (9-11), perinatal asphyxia (7, 12, 13) and antepartum betamethasone treatment (14). Since so many conditions in the newborn are associated with elevated cord blood lipids and lipoproteins, we have investigated: 1) factors during pregnancy and delivery which may affect cord serum lipids; 2) the validity of an easy screening method for detecting hyperlipidemia in the newborn; 3) if in families with familial hypercholesterolemia, examination of cord serum lipids is a reliable way of diagnosing the condition in the infant.

MATERIALS

Cord blood was obtained from 1025 newborn infants born in the Rigshospitalet, Copenhagen. The study and its purpose was explained to the parents and their consent was obtained. The obstetric records of all births were studied without prior knowledge of the cord serum lipid

and lipoprotein values. The data given in Table 1 were collected for each case.

Fetal bradycardia is here defined as: Either a single episode of FHR < 80/min between uterine contractions and/or FHR between 80-100/min during more than two uterine contraction cycles and/or abnormal cardiocardiographic patterns with late deceleration.

The gestational age of low birth weight infants (<2501 g) was assessed by the mother's calculation combined with a clinical examination of the newborn using the technique of Finnstrom (15). In case of uncertainty, the gestational age was further compared with the results of an ultrasonic measurement of the biparietal cranial diameter of the fetus. If the birth weight was below the 10th percentile value for gestation, the infant was classified as SGA.

Abbreviations: FH = familial hypercholesterolemia; AGA = appropriate for gestational age; SGA = small for gestational age; TC = total cholesterol; TG = triglyceride; VLDL = very low density lipoproteins; LDL = low density lipoproteins; HDL = high density lipoproteins; VLDL+LDL = VLDL+LDL determined by turbidimetry; VLDL+LDL-C = VLDL-cholesterol + LDL-cholesterol determined enzymatically after CaCl_2 heparin precipitation; HDL-C = HDL-cholesterol calculated as the difference $(\text{TC}) - (\text{VLDL+LDL-C})$; FHR = fetal heart rate; IUGR = intrauterine growth retardation; AS = Apgar score.

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Table 1 Obstetric data for the 1025 newborns

Maternal edema (demanding treatment with diuretics)	331
Maternal proteinuria	15
Maternal hypertension (130/90 Hg on more than 2 occasions)	37
Maternal diabetes	23 White group A 12 C 4 B 4 D 3
Beta-methasone-phenobarbital-ritodrine treatment	71 AGA >37 <42 weeks 23 AGA >33 <37 weeks 74 SGA >37 <42 weeks 24
Medical induction	139
Medical stimulation	299
Sedatives (within 24 hours before delivery)	240
Analgesics (within 24 hours before delivery)	181
Prolonged labour (>18 hours)	15
Prolonged ruptured membranes (>12 hours)	30
Fever (rectal temperature >38 °C)	2
Placenta praevia	5
Hemorrhage of placenta	6
Infarction of placenta	91
Fetal bradycardia (see text)	94
Abnormal fetal presentation (any other than vertex)	81 (65 breech presentations)
Meconium stained amniotic fluid	112
Firm cord circumflexion	64
Caesarean section	143
Vacuum extraction	135
Forceps delivery	18
AS/1 min <7	68
AS/5 min <7	3
Boys	531
Gestation at age and maturity	896 AGA >37 <42 weeks 47 AGA >33 <37 weeks 2 AGA <33 weeks 68 SGA >37 <42 weeks 12 SGA >33 <37 weeks

In order to prevent RDS betamethasone-phenobarbital-ritodrine treatment was given in 71 cases (Table 1) as described earlier (14). Ritodrine is a beta-adrenergic stimulant which inhibits pre-term uterine contractions. Hereafter this triple drug therapy will be called beta-methasone treatment since all evidence points to this drug as having the powerful effect on lipoprotein synthesis.

Subjects. The umbilical cord was clamped and cut within the first 3 min after birth and prior to delivery of the placenta. Mixed arterial and venous blood was allowed to run freely in dry glass tubes contaminated with maternal blood being carefully avoided. The cord blood was stored at 4 °C for no longer than 12 hours before serum was separated (2000 r.p.m. 20 min) and the analyses begun.

Venous blood samples were taken within the first week after delivery from all 2050 parents without prior fasting. Serum TC was determined and if elevated (>95th sex and age corrected percentile value for normal Danes) (4) and serum cholesterol was determined another three to four times after 12 hours fast and in the mothers at least 3 months after the birth had taken place to make sure that the elevation was permanent and if so the parent and all available family members had their serum lipids and lipoproteins determined.

METHODS

Serum TC was measured manually in duplicate using the enzymatic method described by Roschlau et al. (16). Four Preciset cholesterol standards were included in over 100 separate runs. The coefficient of variation was 4.4% (for the 0.32 mmol/l cholesterol Preciset), 1.7% (for the 1.59 mmol/l cholesterol Preciset), 1.9% (for the 3.88 mmol/l cholesterol Preciset) and 2.1% (for the 10.35 mmol/l cholesterol Preciset).

Serum TG was measured manually in duplicate using the enzymatic method described by Eggstein & Kreutz (17). Two Liponorm triglyceride standards were included in over 100 separate runs. The coefficient of variation was 3.6% (for the 0.42 mmol/l triglyceride Liponorm) and 2.0% (for the 2.50 mmol/l triglyceride Liponorm).

VLDL LDL was measured by a modification of the CaCl_2 Heparin turbidimetric method of Burstein & Samuels (18) on a Greiner Selective Analyzer II using 70 µl of serum and 1000 µl of CaCl_2 Heparin precipitation solution. After 5 min at 37 °C the sample was read at 578 nm against a sample blank. The concentration of VLDL LDL was expressed in arbitrary units corresponding to the extinction A frozen (-20 °C) stock solution of pooled cord serum (VLDL LDL concentration 45 arbitrary units) was

Table 2 Percentile values of cord serum lipids and lipoproteins in AGA and SGA infants after uncomplicated delivery (no betamethasone no asphyxia)

Cord serum	Maturity	No	Cord serum lipid values (mmol/l)						
			2.5	5	10	50	90	95	97.5
T-C	AGA $\geq 37 \leq 42$ weeks	117	1.17	1.31	1.38	1.75	2.49	2.83	3.09
VLDL LDL C	AGA $\geq 37 \leq 42$ weeks	117	0.39	0.43	0.46	0.72	1.03	1.09	1.40
HDL C	AGA $\geq 37 \leq 42$ weeks	117	0.64	0.70	0.76	1.04	1.60	1.74	1.91
TG	AGA $\geq 37 \leq 42$ weeks	117	0.18	0.0	0.23	0.39	0.59	0.70	0.75
T-C	AGA $\geq 33 < 37$ weeks	15			1.19	1.33	1.75		
VLDL LDL C	AGA $\geq 33 < 37$ weeks	15			0.56	0.94	1.58		
HDL C	AGA $\geq 33 < 37$ weeks	15			0.63	1.44	2.21		
TC	AGA $\geq 33 < 37$ weeks	15			0.24	0.30	0.47		
T-C	SGA $\geq 37 \leq 42$ weeks	31			1.26	1.63	2.14		
VLDL LDL C	SGA $\geq 37 \leq 42$ weeks	31			0.55	0.68	1.15		
HDL C	SGA $\geq 37 \leq 42$ weeks	31			0.60	0.93	1.26		
TG ^a	SGA $\geq 37 \leq 42$ weeks	31			0.31	0.55	0.87		

^a $p < 0.05$ compared with AGA $\geq 37 \leq 42$ weeks^b $p < 0.001$ compared with AGA $\geq 37 \leq 42$ weeks

thawed and included in over 100 runs. The coefficient of variance was 1.6%. VLDL LDL C was measured manually after CaCl_2 Hepann precipitation in duplicate as described by Andersen & Gry Nielsen (19). VLDL C was measured manually in duplicate after ultracentrifugation in a 40.3 rotor Beckman type L ultracentrifuge at 10°C at 40000 r.p.m. for 0 hours. Tube slicing was used. HDL C values were calculated as the difference between (T-C) (VLDL LDL C).

Percentile values were calculated to characterize the distribution of the different lipid and lipoprotein-cholesterol values. For comparison of cord serum lipid and lipoprotein-cholesterol values among newborns with a different obstetric history (e.g. differences in gestational age, perinatal drug treatment, perinatal asphyxia) the Mann-Whitney test was used as described by Siegel (20). The relationship between cord serum VLDL LDL C and VLDL LDL was estimated in such a way that the sum of the squared distances from the observations perpendicular to the line was minimized. A test of linearity was carried out by dividing the data into 5 equally sized groups at different levels along the line and by performing a χ^2 test in each group as well as a combined test of all 5 values.

RESULTS

Factors which may affect cord serum lipids
If the 1025 deliveries only 163 were uncomplicated. In Table 2 the distribution of cord serum lipids and lipoproteins of these infants are given.

Cord serum lipids and lipoproteins were compared in 59 normal fullterm boys and 58 normal full term girls. No sex difference was found and so the values for all 117 normal boys and girls are here presented together.

It is seen that T-C and VLDL LDL C levels are higher and TG levels lower in premature newborns (AGA $\geq 33 < 37$ weeks) compared with full term newborns (AGA $\geq 37 \leq 42$ weeks). In small for date infants (SGA $\geq 37 \leq 42$ weeks) HDL C concentrations are lower and TG concentrations higher than in full term infants.

The influence of abnormal pregnancy and birth was studied by comparing the distribution of cord serum lipid and lipoprotein values in the 117 normal full term newborns with the distribution in groups of fullterm newborns, each group having one of the criteria given in Table 1.

In infants who had suffered from perinatal asphyxia serum TG values were higher than in normal newborns. The mean TG and p values are given in Table 3. VLDL LDL C followed a similar pattern.

Betamethasone treatment was accompanied by hypercholesterolemia in full term and premature infants. These results have been presented in detail already (14).

12 newborns of mothers with diabetes mellitus. White group A had a median value for cord serum VLDL LDL C of 0.73 mmol/l which is similar to 0.72 mmol/l in normal newborns. 11 newborns of mothers with diabetes mellitus. White group B-D had a median

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Boys	531
Gestational age and maturity	89% AGA $\geq 37 \leq 42$ weeks 47% AGA $\geq 33 < 37$ weeks 2% AGA < 33 weeks 68% SGA $\geq 37 \leq 42$ weeks 12% SGA < 37 weeks

METHODS

In order to prevent RDS beta-methasone-phenobarbital-ritodrine treatment was given in 71 cases (Table 1) as described earlier (14) (Ritodrine is a beta-adrenergic stimulant which inhibits pre-term uterine contractions). Hereafter this triple drug therapy will be called beta-methasone treatment since all evidence points to this drug as having the powerful effect on lipoprotein synthesis.

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HDL-C	SGA $\geq 37 \leq 42$ weeks	31			0.60	0.93	1.26		
TG	SGA $\geq 37 \leq 42$ weeks	31			0.31	0.55	0.82		

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Of the 1025 deliveries only 163 were uncomplicated. In Table 2 the distribution of cord serum lipids and lipoproteins of these infants are given.

Cord serum lipids and lipoproteins were compared in 59 normal fullterm boys and 58 normal fullterm girls. No sex-difference was found and so the values for all 117 normal boys and girls are here presented together.

It is seen that T-C and VLDL LDL-C levels are higher and TG levels lower in premature newborns (AGA $\geq 33 < 37$ weeks) compared with full term newborns (AGA $\geq 37 \leq 42$ weeks). In small for date infants (SGA $\geq 37 \leq 42$ weeks) HDL-C concentrations are lower and TG concentrations higher than in full term infants.

The influence of abnormal pregnancy and birth was studied by comparing the distribution of cord serum lipid and lipoprotein values in the 117 normal full term newborns with the distribution in groups of fullterm newborns each group having one of the criteria given in Table 1.

In infants who had suffered from perinatal asphyxia serum TG values were higher than in normal newborns. The mean TG and p values are given in Table 3. VLDL LDL-C followed a similar pattern.

Betamethasone treatment was accompanied by hypercholesterolemia in full term and premature infants. These results have been presented in detail already (14).

12 newborns of mothers with diabetes mellitus. White group A had a median value for cord serum VLDL LDL-C of 0.73 mmol/l which is similar to 0.72 mmol/l in normal newborns. 11 newborns of mothers with diabetes mellitus. White group B-D had a median

Table 3 Comparison of triglyceride values in two subgroups of the infants

(A) = normal full term newborns without perinatal asphyxia (B) = full term newborns with signs of perinatal asphyxia

Criteria	No	TG median value (mmol/l)	p Value
(A) Normal newborns	117	0.39	
(B) Newborns with perinatal asphyxia			
1 Late decelerations	15	0.67	<0.001
2 FHR <80/min	29	0.57	<0.001
3 FHR <120>80/min	34	0.49	<0.001
4 Meconium stained amniotic fluid	92	0.46	<0.001
5 Firm cord circumflexion	57	0.46	<0.005
6 Vacuum extraction	118	0.46	<0.001
7 Apgar 1 min <7	41	0.45	<0.05

value of 0.95 mmol/l which is significantly higher than in normal newborns ($p < 0.005$). This finding however must be interpreted with great caution since the number is small 4 of the 11 were prematurely born and 1 was SGA.

The influence of postmaturity could not be investigated since none of the 1025 newborns were born after the 42nd week of gestation.

All of the other criteria presented in Table 1 i.e. maternal edema, proteinuria, hypertension, infarction and hemorrhage of placenta, prolonged ruptured membranes, prolonged labour, medical induction and stimulation

sedatives, analgesia, abnormal fetal presentation, Caesarean section and forceps delivery did not significantly influence the levels of cord serum lipids and lipoproteins.

The screening method In Fig 1 the log transformed values of 1025 cord serum VLDL, LDL, C are plotted against the log transformed values of cord serum VLDL, LDL. It is seen that all specimens belonging to the upper 5 percent for VLDL, LDL, C will be included when the upper 15% VLDL, LDL values are selected for further study. Thus of the 154 newborns in this study with a VLDL, LDL concentration above the 85th percentile

Table 4 Lipid and lipoprotein cholesterol values (mmol/l) in parent child

Kindred	Age (years)	T C	VLDL LDL C	LDL C	HDL	TG
1 II 8	28	8.97	6.35	6.19	2.62	0.67
1 III 9	At birth	2.05	1.12	1.93	1.93	0.77
1 III 9	1 1/12	4.79	3.54	3.30	1.25	0.84
2 III 1	32	9.82	7.75	7.25	2.07	1.74
2 IV 1	At birth	1.70	0.77	0.93	0.93	0.63
2 IV 1	1 1/12	2.80	1.59	1.45	1.21	0.85
3 II 1	31	8.72	6.08	5.71	2.64	1.07
3 III 2	At birth	1.49	0.59	0.90	0.90	0.31
3 III 2	1 1/12	2.80	1.50	1.15	1.30	0.86
4 II 7	23	9.74	8.62	8.42	1.12	0.89
4 III 2	At birth	2.59	1.38	1.21	1.21	0.67
4 III 2	1 1/12	4.70	3.11	2.87	1.59	1.31
5 III 3	33	9.89	7.45	6.80	2.44	1.79
5 IV 2	At birth	1.90	0.75	1.15	1.15	0.41
5 IV 2	1 1/12	6.59	4.94	4.38	1.65	1.67

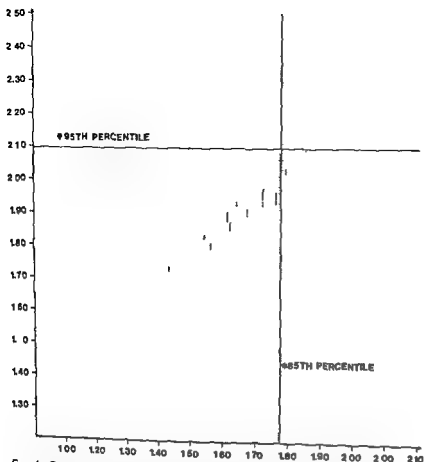


Fig 1 Correlation between 1075 log values for (cord serum VLDL LDL C $\times 100$) (ordinate) and log values for (cord serum VLDL LDL $\times 100$) (abscissa). Three VLDL LDL C values fell on the 95th percentile. To visualize

these 3 points they have been put above the 95th percentile line $\log (\text{VLDL LDL C}) = 0.91 \times \log (\text{VLDL LDL}) + 0.394$

47 had VLDL LDL C values above the 95th percentile. Follow up values of these 47 infants have been presented in another publication (21).

Diagnosing FH at birth Among the 1025 fathers 55 had hypercholesterolemia (defined as above 95 percentile value for serum TC in normal Danish men (4)) and among the 1025 mothers 137 had hypercholesterolemia within the first week after delivery (defined as above 95 percentile value for serum TC in normal Danish women (4)). These 192 parents were followed and their serum TC was measured another 2-3 times and at least 3 months after delivery. In only 3 fathers and 2 mothers a permanent pronounced hypercholesterole-

mia (over 7.80 mmol/l) was found which gave a suspicion of FH. The diagnosis of heterozygous FH is here based upon the finding of a 2-3 fold increase in serum TC and/or LDL C either vertically in three generations or in a family with xanthomatosis. The 5 kindreds are depicted in Fig 2 and the corresponding lipid values are given in Table 4. In kindred 1 the child does not have FH. His lipids were normal at birth, 5 months and 1 1/2 years. His father has FH with a type II a pattern. In kindred 2 the child does not have FH. Her lipids were normal at birth, 1 month and 1 1/2 years. Her father has FH with a type II a pattern. In kindred 3 the child does not have FH. His lipids were normal at birth, 5 months

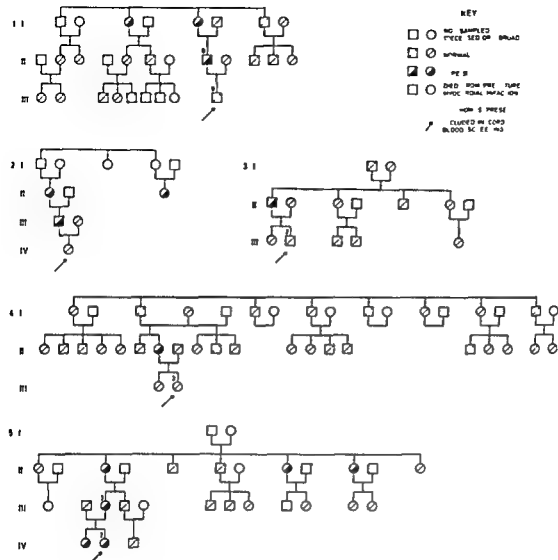


Fig. 2 Kindreds

and 1½ years. His father has a type II a pattern but he does not have FH since his parents are normal. Common causes of secondary hyperlipemia were ruled out by repeated findings of normal glucose tolerance test, normal liver, thyroid and kidney function tests and normal immunoglobulins. In kindred 4 the child does not have FH. Her cord serum VLDL LDL C was 1.38 mmol/l, which is above the 95th percentile; her cord serum TC, however, was normal = 2.59 mmol/l. Her lipoprotein and lipid values were normal at 3 months, 1½ and 1½ years. Her mother has a type II a pattern, but she does not have FH. Also in this case common causes of secondary hyperlipemia were ruled out. In kindred 5 the child has FH with elevated lipids at age 3

months, 1½ and 1½ years. Her cord serum TC and VLDL LDL C, however, were normal. Her mother has FH with a type II a pattern. The mother developed abnormal glucose tolerance during pregnancy, which again became normal after delivery.

DISCUSSION

In our 117 normal newborns, the median concentrations of cord serum TC, VLDL LDL C, HDL C and TG were almost similar to the mean concentrations found by Glueck et al (22), Kwiterovich et al (1), Ose et al (23) and Andersen & Friis-Hansen (6). With one exception (22), the 95th percentile cut-off value for cord serum VLDL LDL C seems to be be

tween 1.09–1.27 mmol/l for normal newborns. The present finding of higher than normal cord serum T C, VLDL, LDL C and HDL C values in premature newborns (≥ 33 – < 37 weeks of gestation) confirms the results of Sabata (24), Ose et al. (23) and Andersen & Fris Hansen (6) but is different from the results obtained by Fosbrooke & Wharton (8) who in 16 premature newborns (28–36 weeks of gestation) found the same levels of cord serum T C as in full term newborns. The reason for this might be their inclusion of premature newborns of under 33 weeks of gestation. We suggest that during the maximal growth rate period between the 33rd and 37th week of gestation during which the fetus will increase its weight by about 200 g/week the need of particularly hepatic VLDL, LDL C for membrane and hormone synthesis by non hepatic parenchymal cells is increased. During the decelerating growth rate period after the 37th week of gestation during which the weekly increase in fetal weight averages only 70 g, the serum concentration of T C, VLDL, LDL C and HDL C falls corresponding to lower cord serum values in full term compared to premature newborns. Our finding of elevated cord serum TG in SGA infants born after IUGR is in agreement with the results of Fosbrooke & Wharton (8) and Andersen & Fris Hansen (6). Also do our findings of elevated TG and VLDL, LDL C in newborns with perinatal asphyxia confirm the results reported by Tsang et al. (12), Andersen & Fris Hansen (13) and Cress et al. (7).

In an earlier publication (19) we have shown that there is a good correlation between the concentration of cholesterol in VLDL+LDL isolated in the ultracentrifuge and in VLDL, LDL after heparin CaCl_2 precipitation. In the present study a good linear correlation was found between VLDL, LDL C and VLDL, LDL thus indicating that for screening purposes the rapid and inexpensive turbidimetric determination of VLDL, LDL can be used instead of the more laborious and expensive measurement of VLDL, LDL C. The draw-

back of the screening method is the necessity to include as much as 15% of the specimens in a more elaborate analytical procedure to be sure to select the 5% with the highest VLDL, LDL C values.

The possible diagnosis of FH in newborns has been a matter of debate within the last few years. The interest in making an early diagnosis has to do with the theoretical benefit in reducing the increased risk of premature coronary heart disease by early dietary and pharmaceutical intervention. Five studies (1, 2, 4, 25, 26) have shown that measurement of cord serum T C alone does not discriminate newborns with FH. This is probably due to the fact that most of the cord serum T C is not transported by the LDL but by the HDL fraction. Four studies (1–4) have shown that an elevation of cord serum LDL or VLDL+LDL will give a suspicion of FH in the child but only one study (1) has unambiguously shown that an elevated cord serum LDL C combined with a follow up study after year one in a child with known FH in one parent allows a diagnosis of FH. In children with unknown parental phenotypes the three existing studies have been incomplete and inconclusive as for estimation of incidence of FH and false positive and negative diagnosis. Greten et al. (2) in 1323 children found 11 (0.45%) having an elevated VLDL, LDL C at birth and after age one but the diagnosis FH was not verified since no family studies were carried out. Tsang et al. (3, 22) in 1800 consecutively born infants found 3 (0.17%) having an elevated (>95th percentile value) LDL C at birth and after age one combined with a three generation vertical transmission of elevated T C in one parent and grandparent. However only newborns with an elevated T C had their cord serum LDL C determined. Andersen & Fris Hansen (4) among 303 full term newborns found 3 (0.99%) with an elevated (>97.5th percentile) cord serum prebeta betalipoprotein value who at follow up after year one and by three generation vertical family studies were shown to have FH. This study however did

not include measurement of cord serum VLDL LDL C and the newborns were all full term

In the present study of 1025 infants 1 child (0.10%) had FH but this diagnosis was not suspected at birth since her cord serum VLDL LDL C was normal. The reason why she did not have elevated VLDL LDL C at birth is unclear. The abnormal glucose tolerance found in the mother just prior to birth could hardly explain this finding of normal cord serum lipids. Glueck (27) also has studied a child with FH who had normal cord serum lipid and lipoprotein values of which no explanation could be given.

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REFERENCES

- 1 Kwitterovich P O, Levy R I & Fredrickson D S Neonatal diagnosis of familial type II hyperlipoproteinemia *Lancet* 1 118 1973
- 2 Greten H, Wigner M & Scheitler G Frühdiagnose und Häufigkeit der familiären Hyperlipoproteinämie Typ II *Dtsch Med Wochenschr* 99 2553 1974
- 3 Tsang R C, Glueck C J, Jallat R W & Mellies M Neonatal familial hypercholesterolemia *Am J Dis Child* 129 83 1975
- 4 Andersen G E & Fris Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics* 57 214 1976
- 5 Glueck C J, Fallot R W, Millett F & Steiner P M Familial hyperalphalipoproteinemia *Acta Int Med* 135 1025 1975
- 6 Andersen G E & Fris Hansen B Cord serum lipid and lipoprotein cholesterol values in normal and beta methasone treated newborns of varying gestational age *Acta Paediatr Scand* 66 355 1977
- 7 Cress H R, Shafer R M, Laffin R & Karpowicz K Cord blood hyperlipoproteinemia and perinatal stress *Jediatr Res* 11 19 1977
- 8 Fosbrooke A S & Wharton B A Plasma lipids in umbilical cord blood from infants of normal and low birth weight *Biol Neonat* 23 330 1973
- 9 Mortimer J G Cord blood lipids of normal infants and infants of diabetic mothers *Arch Dis Child* 39 342 1964
- 10 Pantelakis N, Cameron A H, Davidson S, Dunn P M, Fosbrooke A S, Lloyd J K, Malins J M & Wolff O H The diabetic pregnancy A study of serum lipids in maternal and umbilical cord blood and of the uterine and placental vasculature *Arch Dis Child* 39 334 1964

- 11 Lloyd A V Observations on the composition of the blood in the neonatal period Thesis University of Edinburgh 1963
- 12 Tsang R C, Glueck C J, Evans G & Steiner P M Cord blood hypertriglyceridemia *Am J Dis Child* 127 78 1974
- 13 Andersen G E & Fris Hansen B Neonatal hypertriglyceridemia *Acta Paediatr Scand* 65 369 1976
- 14 Andersen G E & Fris Hansen B Hypercholesterolemia in the newborn after antepartum treatment with betamethasone phenobarbital nitrodrine for the prevention of the respiratory distress syndrome *Pediatrics* 62 8 1978
- 15 Finnström O Studies on maturity in newborn infants II External characteristics *Acta Paediatr Scand* 61 24 1972
- 16 Roschlau P, Berni E & Gruber W Enzymatische Bestimmung des Gesamt Cholesterins im Serum *Z Klin Chem Klin Biochem* 17 403 1974
- 17 Eggstein M & Kreuz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe *Klin Wochenschr* 44 262 1966
- 18 Burstein M & Samaille J Nouvelle methode de separation et de dosage des lipoproteines de faible densité *Ann Biol Clin* 17 23 1959
- 19 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL+LDL *Clin Chim Acta* 66 29 1976
- 20 Siegel S In S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 p 116
- 21 Andersen G E, Lous P & Fris Hansen B Screening for hyperlipoproteinemia in 10000 Danish newborns Follow up studies in 97 children with elevated cord serum VLDL LDL cholesterol *Acta Paediatr Scand* 68 541 1979
- 22 Glueck C J, Heckman F, Schoenfeld M, Steiner P & Pearce W Neonatal familial type II hyperlipoproteinemia Cord blood cholesterol in 1800 births *Metabolism* 20 597 1971
- 23 Ose L, Brakke T, Iden A & Aarskog D Neonatal screening for hyperlipidaemia *Postgrad Med J* 51 (Suppl 8) 89 1975
- 24 Šabata V Carbohydrate and lipid metabolism of the human fetus *Ro.pra v Československe Akademie Ved* 83 9 1973
- 25 Dirmady J M, Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during first year of life *Br Med J* 11 685 1972
- 26 Potter J M & Nestel P J Comparison of plasma lipids at birth and in second year of life *Aust N Z J Med* 6 420 1976
- 27 Glueck C J Personal communication

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PROSPECTIVE STUDIES OF THE EFFECT OF BREAST FEEDING ON INCIDENCE OF INFECTION AND ALLERGY

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ABSTRACT Chandra R K (Department of Pediatrics Memorial University of Newfoundland St John's Newfoundland Canada) Prospective studies of the effect of breast feeding on incidence of infection and allergy *Acta Paediatr Scand* 68 691 1979 —The effect of exclusive breast feeding in the first few weeks after birth on infant morbidity due to infectious and allergic disorders was investigated in three separate prospective studies. In a rural community in India breast fed infants had a significantly lower incidence of respiratory infection otitis diarrhoea dehydration and pneumonia. In an urban population in Canada breast feeding was associated with a marked decrease in the occurrence of otitis and respiratory disease and to a lesser extent of diarrhoea and dehydration. In newborn siblings of children with atopic disease exclusively breast fed for a minimum of six weeks the incidence of eczema recurrent wheezing elevated serum IgE IgE-antibodies to cow's milk complement activation *in vivo* after milk challenge and hemagglutinating antibodies to β -lactoglobulin was significantly lower compared with formula fed matched group. These observations provide clinical data attesting the immunologic advantages of human milk.

KEY WORDS Breast feeding human milk infection allergic disease morbidity eczema asthma immunoglobulin E.

The deleterious effects of artificial feeding techniques supplanting breast feeding have been documented repeatedly. Microbial colonization and infection related morbidity is reported to be lower in infants fed human milk compared with those fed on cow's milk formula (6 10-12 21 25 28 29). However many of the studies showing health advantage for breast fed infants relied on anecdotal clinical experience medical records and retrospective data (see 6). Recently elaborate demonstration of a variety of protective factors in human milk has provided sound immunologic evidence of the beneficial effects of breast feeding. In this paper controlled prospective observations indicate that breast feeding is associated with a reduction in the incidence of infections and of allergy.

MATERIAL AND METHODS

Infants with related morbidity

In a Thirty five newborn infants fed exclusively on the breast for at least the first 2 months of life were studied

The average duration of exclusive breast feeding was 4.8 months (range 2-8.5 months). The age upto which breast feeding was continued ranged from 4 to 24 months (mean 9.5 months). A trained auxiliary nurse midwife visited each family once a week and recorded morbidity data. Thirty five controls bottle fed on fresh cow's or buffalo's milk from the first week onwards were similarly observed. The two groups were matched for socioeconomic status parental education and occupation and family size. Respiratory infection was defined as cough during the day and night for 7 hours or more with or without fever or coryza. Otitis was defined as mucopurulent discharge from external auditory meatus with or without fever irritability and pulling at ears. Diarrhoea was defined as 3 or more loose bowel movements for 48 hours or longer. Dehydration was diagnosed on physical examination and pneumonia by radiology.

Canada: Thirty neonates fed exclusively on the breast for at least the first two months of life were followed up for a period of 24 months. The average duration of exclusive breast feeding was 3-6 months (range 2.5-5.8 months). The age up to which breast feeding was continued ranged from 4 to 14 months (mean 7.5 months). Thirty infants fed on cow's milk formula (Carnation) from the first day onwards acted as controls. The two groups were matched for socioeconomic status parental education and family size. The parents were contacted by telephone once in two weeks and morbidity data recorded. In most instances the diagnosis of illness was confirmed on physical examination.

not include measurement of cord serum VLDL LDL C and the newborns were all full term

In the present study of 1025 infants 1 child (0.10%) had FH but this diagnosis was not suspected at birth since her cord serum VLDL LDL C was normal. The reason why she did not have elevated VLDL LDL C at birth is unclear. The abnormal glucose tolerance found in the mother just prior to birth could hardly explain this finding of normal cord serum lipids. Glueck (27) also has studied a child with FH who had normal cord serum lipid and lipoprotein values of which no explanation could be given.

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REFERENCES

- 1 Kwaterovich P O, Levy R I & Fredrickson D S Neonatal diagnosis of familial type II hyperlipoproteinemia *Lancet* 1 118 1973
- 2 Greten H, Wagner M & Schettler G Frühdiagnose und Häufigkeit der familiären Hyperlipoproteinnämie Typ II *Dtsch Med Wochenschr* 99 2553 1974
- 3 Tsang R C, Glueck C J, Fallat R W & Mellies M Neonatal familial hypercholesterolemia *Am J Dis Child* 129 83 1975
- 4 Andersen G E & Fris Hansen B Neonatal diagnosis of familial type II hyperlipoproteinemia *Pediatrics* 57 214 1976
- 5 Glueck C J, Fallat R W, Millett F & Steiner P M Familial hyperlipoproteinemia *Acta Int Med* 135 1025 1975
- 6 Andersen G E & Fris Hansen B Cord serum lipid and lipoprotein cholesterol values in normal and beta methasone treated newborns of varying gestational age *Acta Paediatr Scand* 66 355 1977
- 7 Cress H H, Shaher M M, Laffin M & Karpowicz K Cord blood hyperlipoproteinemia and perinatal stress *Pediatr Res* 11 19 1977
- 8 Fosbrooke A M & Wharton B A Plasma lipids in umbilical cord blood from infants of normal and low birth weight *Biol Neonate* 23 330 1973
- 9 Mortimer J G Cord blood lipids of normal infants and infants of diabetic mothers *Arch Dis Child* 39 342 1964
- 10 Pantelakis N N, Cameron A H, Davidson S, Dunn P M, Fosbrooke A S, Lloyd J K, Malins J M & Wolff O H The diabetic pregnancy A study of serum lipids in maternal and umbilical cord blood and of the uterine and placental vasculature *Arch Dis Child* 39 334 1964
- 11 Lloyd A V Observations on the composition of the blood in the neonatal period Thesis University of Edinburgh 1963
- 12 Tsang R C, Glueck C J, Evans G & Steiner P M Cord blood hypertriglyceridemia *Am J Dis Child* 127 78 1974
- 13 Andersen G E & Fris Hansen B Neonatal hypertriglyceridemia *Acta Paediatr Scand* 65 369 1976
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- 16 Roschlau P, Berni E & Gruber W Enzymatische Bestimmung des Gesamt Cholesterins im Serum *Z Klin Chem Klin Biochem* 12 403 1974
- 17 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe *Klin Wochenschr* 44 262 1966
- 18 Burstein M & Samaille J Nouvelle methode de separation et de dosage des lipoproteines de faible densite *Ann Biol Clin* 17 23 1959
- 19 Andersen G E & Gry Nielsen H Neonatal screening for hyperlipoproteinemia Methods for direct estimation of cord serum VLDL+LDL *Clin Chim Acta* 66 29 1976
- 20 Siegel S In S Siegel (ed) *Nonparametric statistics for the behavioral sciences* McGraw Hill New York 1956 p 116
- 21 Andersen G E, Lous P & Fris Hansen B Screening for hyperlipoproteinemia in 10000 Danish newborns Follow up studies in 92 children with elevated cord serum VLDL LDL cholesterol *Acta Paediatr Scand* 68 541 1979
- 22 Glueck C J, Heckman F, Schoenfeld M, Steiner P & Pearce W Neonatal familial type II hyperlipoproteinemia Cord blood cholesterol in 1800 births *Metabolism* 20 597 1971
- 23 Ose L, Bakke T, Iden A & Aarskog B Neonatal screening for hyperlipidaemia *Postgrad Med J* 51 (Suppl 8) 88 1975
- 24 Šabrita V Carbohydrate and lipid metabolism of the human fetus *Rozprawy Československe Akademie Ved* 83 9 1973
- 25 Darmady J M, Fosbrooke A S & Lloyd J K Prospective study of serum cholesterol levels during first year of life *Br Med J* 11 685 1972
- 26 Potter J M & Nestel P J Comparison of plasma lipids at birth and in second year of life *Aust N Z J Med* 6 420 1976
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Table 3 Incidence of eczema, recurrent wheezing and *in vitro* correlates of allergy in breast fed and formula fed infants

Figures refer to the number of infants affected or showing positive test

Parameter	Breast fed (n=37)	Formula fed (n=37)	P
Eczema	4	21	<0.001
Recurrent wheezing	1	8	<0.05
Serum IgE >60 IU/ml	6	29	<0.001
IgE antibodies to cow's milk	1	15	<0.001
Complement activation <i>in vivo</i> after milk challenge	0	6	<0.001
Hemagglutinating antibodies to β lactoglobulin	3	31	<0.05
Eosinophilia 400 per mm ³	0	5	<0.05

been detected and are functionally effective in the gut lumen against the respective micro organisms and their products (15, 18, 20, 24). There is recent evidence to suggest that colostral antibodies can be absorbed by the healthy newborn for the first three days after birth and by the low birth weight infant for several weeks (R. K. Chandra unpublished data). The antibody content of milk reflects the immunologic experience of the mother's gastrointestinal tract (19) and may be mediated by unique characteristics of lymphocyte traffic in the enteric circulation and lymphatics. T and B lymphocytes, neutrophils and macrophages are present and immunocompetent (3, 6, 24, R. K. Chandra unpublished data). Furthermore, antigen nonspecific resistance factors such as lactoferrin, lysozyme and interferon provide additional antimicrobial activity in breast milk (1, 14). These many unique properties of human milk provide distinct advantages to the breast fed infant in terms of morbidity, experience and survival, particularly when children are raised in less than ideal circumstances (4, 8). Additionally, nutritional deprivation due to poverty and contamination of the formula by enteropathogens as the result of poor hygiene and inadequate cleaning and storage facilities may increase the chances of gastrointestinal infections in the bottle fed infant.

The protective effect of breast feeding in the prevention of allergy was clearly established in our study. Differences in incidence of clinical

atopic disease and complementary laboratory evidence in the breast fed and artificially fed groups (Table 3) strongly incriminate cow's milk formula in the genesis of hypersensitivity in susceptible infants. This lends further support to our previous data (5, 7) and recent reports of other studies (13). Mathew et al. (22) followed up infants of atopic parents and observed a dramatic reduction in the incidence of clinical eczema in the group exclusively breast fed for the first 12 weeks of life. Positive skin reactions to environmental inhalants and ingestants were seen less frequently than in the formula fed control group. The increased permeability of the neonatal intestine to dietary macromolecules provides an avenue for initial sensitization to food allergens. The duration of the postnatal period of such susceptibility is not established and may well be only a few weeks, differing in healthy and growth retarded infants and reappearing in the older infant during the acute and convalescent phases of viral gastroenteritis (R. K. Chandra unpublished data). The subject of breast feeding and common atopic disease has been reviewed elsewhere (6, 27). The literature suggests that exclusive breast feeding will reduce the incidence of allergy in the at risk infant. Our data shows that six weeks of exclusive breast feeding is effective in unmasking the development of hypersensitivity and in lowering the incidence of manifest allergic disease in vulnerable infants.

Table 1 *Infection related morbidity in breast fed and formula fed infants in India*

The data is expressed as number of episodes of illness for the group over a 12 month period n =number of infants in each group. Differences between the two groups were analyzed by χ^2 test

Disorder	Breast fed ($n=35$)	Formula fed ($n=35$)	p
Respiratory infection	57	109	<0.001
Otitis	21	52	<0.001
Diarrhoea	70	211	<0.001
Dehydration	3	14	<0.001
Pneumonia	2	8	<0.001

Allergy

Thirty seven infants with an older sibling diagnosed to have atopic disease were exclusively fed on the breast for the first six weeks of life or longer. During this period no supplements other than water were given. Each infant was examined at 3 month intervals during the first year and thereafter at 6-month intervals till the age of 3 years. All children were followed up for a minimum of 24 months (median 27.8 months). Another group of 37 infants who also had an older sibling with allergic disease but were fed cow's milk formula were similarly examined. The two groups did not differ significantly in any other respect. Identical advice concerning the nature and the age of introduction of food supplements was offered to all the families. Blood was collected at the ages of 12 and 24 months. IgE was estimated by radioimmunoassay (Pharmacia) IgE antibodies by the radioallergen sorbent test (Pharmacia) and antibodies to β lactoglobulin by tanned red cell hemagglutination (2). In addition evidence for complement activation after cow's milk challenge was sought by immunoelectrophoresis and complement C3 concentration (23). Eczema was diagnosed on the basis of characteristic distribution and morphology of skin lesions. Wheezing was considered to be of allergic etiology if 3 or more episodes had occurred, absence of fever at onset and dramatic response to sympathomimetic agents like adrenaline and orciprenaline.

RESULTS

There was a significant difference in infectious morbidity among breast fed and bovine milk fed infants both in India (Table 1) and in Canada (Table 2). There was a lower incidence of respiratory and diarrhoeal disease and of complications such as pneumonia and dehydration. Among urban Canadian infants there was a dramatic beneficial effect of breast feeding on occurrence of middle ear infection, a reduction of almost 10 fold was observed.

In infants with family history of atopy exclusive breast feeding for a period of 4 weeks or longer was associated with a marked reduction in the incidence of clinical atopic eczema and of recurrent allergic wheezing (Table 3). A larger proportion of formula fed infants had high levels of serum IgE. Eosinophilia was seen in 6 of 37 artificially fed infants and in none of the breast fed babies. IgE antibodies to cow's milk proteins were detected in 40% of the formula fed group and in 3% of the breast fed group and hemagglutinating antibodies to β lactoglobulin were observed in 84% and 8% respectively. Complement activation *in vivo* after milk challenge was found only in some of the formula fed infants.

DISCUSSION

Our controlled prospective studies, one in a developing country and the other in an industrialized country, have confirmed the significant beneficial effects of breast feeding on infection related morbidity. Many of the previous reports critically analyzed elsewhere (6) were based on retrospective data and had failed to provide incontrovertible evidence on the subject. Many different proteins, enzymes and cells constitute host resistance factors in the human colostrum and milk (6, 9, 14, 17, 19, 26). Secretory antibodies against a variety of viruses, enterobacteria and enterotoxins have

Table 2 *Infection related morbidity in breast fed and formula fed infants in Canada*

The data is expressed as number of episodes of illness for the group over a 24 month period n =number of children in each group. Differences between the two groups were analyzed by χ^2 test

Disorder	Breast fed ($n=40$)	Formula fed ($n=30$)	p
Respiratory infection	42	98	<0.001
Otitis	9	86	<0.001
Diarrhoea	5	16	<0.01
Dehydration	0	3	0.05 < p < 0.1

SERUM IgA IN THE NEONATE

Molecular Size Concentration and Effect of Breast Feeding

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Blood Transfusion Service Royal Infirmary Edinburgh Scotland*

ABSTRACT Yap P L Pryde A Latham P J and McClelland ■ B L (MRC Unit of Reproductive Biology 2 Forrest Road Edinburgh Department of Therapeutics Edinburgh University Neonatal Unit Simpson Memorial Maternity Pavilion and Blood Transfusion Service Royal Infirmary Edinburgh) Serum IgA in the neonate Molecular size concentration and effect of breast feeding *Acta Paediatr Scand* 68 695 1979.—IgA concentrations in the serum of 48 six day-old neonates (23 exclusively artificially fed 25 exclusively breast fed) were measured using a double antibody radioimmunoassay In 24 of the neonates umbilical cord blood was also studied Gel filtration was used to estimate the molecular size of IgA present in cord and neonatal serum The arithmetic mean concentration of IgA (\pm S.E.M.) found in the 48 neonates was 2.6 ± 1.45 mg/l No significant difference was detected between the breast fed and artificially fed neonates Only 7S IgA was detected in cord blood and in neonatal serum Six days of exclusive breast feeding therefore has no influence on total serum IgA levels on the sixth day of neonatal life nor does it result in detectable circulating 11S IgA at that time

KEY WORDS Serum IgA 6-day-old neonate breast feeding

The human Immunoglobulin A system is immature at birth (15) However the breast fed neonate receives by mouth large quantities of secretory IgA in the form of colostrum and milk (8 12) The IgA in colostrum and milk differs from the IgA found in adult serum consisting largely of a dimer with a sedimentation coefficient of 11S and molecular weight of 370 000 compared with serum IgA which is a 7S monomer of molecular weight 170 000 (5 9) This is mainly due to the presence of an additional protein secretory component which in combination with two molecules of monomeric IgA results in a dimer that is more resistant to digestion by intestinal proteolytic enzymes (2) Although it is known that the majority of IgA in feeds of colostrum and milk is not absorbed by the infant (1 7 10) it has been suggested that the gut in early neonatal life is capable of absorbing small quantities of

IgA (6 10) However the assay techniques which have been used have been relatively insensitive and it was considered desirable to examine this question using a very sensitive assay in a study of normally breast fed infants

The purpose of this study was to observe if exclusive breast feeding for 1 week produced a detectable effect on serum IgA levels in the neonate We have compared by means of a specific IgA radioimmunoassay the serum IgA concentrations in neonates after six days of exclusive breast feeding with serum IgA concentrations in neonates that were exclusively artificially fed In addition we have studied the metabolism of IgA in early life by analysing cord blood samples and comparing IgA concentrations present at birth with those found in the same infant six days later We have also estimated by gel filtration the molecular size of IgA in the neonate

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REFERENCES

- 1 Bullen J J, Rogers H J & Leigh I. Iron binding protein in milk and resistance to *Escherichia coli* in feces in infants. *Br Med J* 1 69 1972
- 2 Chandra R K. Food antibodies in malnutrition. *Arch Dis Child* 50 532 1975
- 3 Chandra R K. Nutrition, dietary factors and immunology of the gut. In *Tripartite Symposium on Gastroenterology*. City University London 1977
- 4 Chandra R K. Basis for possible interventions to improve the immune response in the malnourished. In *International Symposium on Nutritional Interventions*. Instituto de Nutrición y Tecnología de los Alimentos. Universidad de Chile. Santiago 1978
- 5 Chandra R K. Breast feeding and prevention of atopic eczema. *Pediatr Res* 1 P 478 1978
- 6 Chandra R K. Immunological aspects of human milk. *Nutr Rev* 36 265 1978
- 7 Chandra R K. Nutritional deficiency and susceptibility to infection. *Bull WHO* 57 167 1979
- 8 Chandra R K & Newberne P M. *Nutrition, immunity and infection. Mechanisms of interactions*. Plenum New York 1977
- 9 Chandra R K, Mandeville R P & Dearlove J. What's new in clinical immunology. In L S Prasad (ed.) *Paediatric Problems in South East Asia*. Sanjivan Press, Patna 1977 p. DI
- 10 Crosse V M. *The premature baby*. Churchill London 1966 p. 143
- 11 Cunningham A S. Morbidity in breast fed and artificially fed infants. *J Pediatr* 90 726 1977
- 12 Downham M A P, Scott Sims D G, Webb J K G & Gardner P S. Breast feeding protects against respiratory syncytial virus infections. *Br Med J* 11 274 1976
- 13 Editorial. Towards prevention of allergy. *Lancet* 1 339 1977
- 14 Goldman A S & Smith C W. Host resistance factors in human milk. *J Pediatr* 87 1082 1973
- 15 Gotheffors L. Studies of antimicrobial factors in human milk and bacterial colonization of the newborn. Medical dissertation. Umeå Universitet Umeå 1975
- 16 Gotheffors L, Olling S & Winberg J. The effect of human milk on the intestinal flora. Relevance for host resistance. In *Swedish Nutrition Foundation Symposium on Food and Immunology*. Almquist & Wiksell Stockholm 1977 p. 154
- 17 Hanson L Å & Winberg J. Breast milk and defense against infection in the newborn. *Arch Dis Child* 47 845 1972
- 18 Hanson L Å, Ahlstedt B, Carlsson B, Goldblum R M, Lindblad B S & Kayser B. The antibodies of hum in milk: their origin and specificity. In *Swedish Nutrition Foundation Symposium on Food and Immunology*. Almquist & Wiksell Stockholm 1977 p. 148
- 19 Hanson L Å, Ahlstedt B, Carlsson B, Fällström S P, Kayser B, Lindblad B S, Söhl Åkerlund A & Svanborg Eden C. New Knowledge in human milk immunology. *Acta Paediatr Scand* 67 577 1978
- 20 Holmgren J, Hanson L Å, Carlsson B, Lindblad B S & Rahimtoola J. Neutralizing antibodies against *Escherichia coli* and *Vibrio cholerae* enterotoxins in human milk in a developing country. *Scand J Immunol* 5 867 1976
- 21 Mata L J & Urrutia J J. Intestinal colonization of breast fed children in a rural area of low socioeconomic level. *Ann N Y Acad Sci* 176 93 1971
- 22 Mithew D J, Taylor B, Norman A P, Turner M W & Soothill J F. Prevention of eczema. *Lancet* 1 321 1977
- 23 Matthews T S & Soothill J F. Complement activation after milk feeding in children with cow's milk allergy. *Lancet* 11 893 1970
- 24 Ogra S S & Ogra P L. Immunologic aspects of human colostrum and milk. *J Pediatr* 97 446 1978
- 25 Plank S J & Milanesi M L. Infant feeding and infant mortality in rural Chile. *Bull WHO* 48 61 1973
- 26 Reddy V, Bhaskaram C, Raghuramulu N & Jagadeesan V. Antimicrobial factors in human milk. *Acta Paediatr Scand* 66 229 1977
- 27 Soothill J F. Immunodeficiency, allergy and infant feeding. In *Swedish Nutrition Foundation Symposium on Food and Immunology*. Almquist & Wiksell Stockholm 1977 p. 83
- 28 Trissovatz B & Kotsch A. Le lait de femme et son action de protection contre les infections intestinales chez le nouveau né. *Ann Pediatr* 37 1649 1961
- 29 Winberg J & Wessner G. Does breast milk protect against septicaemia in the newborn? *Lancet* 1 1091 1971

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The human Immunoglobulin A system is immature at birth (1). However the breast fed neonate receives by mouth large quantities of secretory IgA in the form of colostrum and milk (8, 12). The IgA in colostrum and milk differs from the IgA found in adult serum consisting largely of a dimer with a sedimentation coefficient of 11S and molecular weight of 370 000 compared with serum IgA which is a 7S monomer of molecular weight 170 000 (5, 9). This is mainly due to the presence of an additional protein secretory component which in combination with two molecules of monomeric IgA results in a dimer that is more resistant to digestion by intestinal proteolytic enzymes (2). Although it is known that the majority of IgA in feeds of colostrum and milk is not absorbed by the infant (1, 7, 10) it has been suggested that the gut in early neonatal life is capable of absorbing small quantities of

IgA (6, 10). However the assay techniques which have been used have been relatively insensitive and it was considered desirable to examine this question using a very sensitive assay in a study of normally breast fed infants.

The purpose of this study was to observe if exclusive breast feeding for 1 week produced a detectable effect on serum IgA levels in the neonate. We have compared by means of a specific IgA radioimmunoassay the serum IgA concentrations in neonates after six days of exclusive breast feeding with serum IgA concentrations in neonates that were exclusively artificially fed. In addition we have studied the metabolism of IgA in early life by analysing cord blood samples and comparing IgA concentrations present at birth with those found in the same infant six days later. We have also estimated by gel filtration the molecular size of IgA in the neonate.

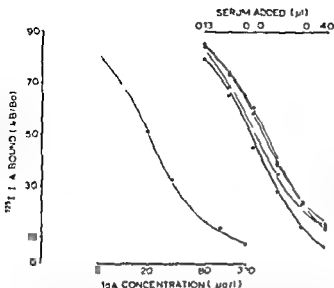


Fig. 1 Inhibition curves for serum IgA standard (■—■) cord blood (▲—▲ and △—△) six day heel prick serum from an exclusively artificially fed neonate (○—○) and six day heel prick serum from an exclusively breast fed neonate (●—●).

MATERIALS AND METHODS

Sera

Forty eight healthy full term neonates were studied twenty three of the neonates were exclusively artificially fed and 25 were exclusively breast fed. Cord blood was collected from 24.

On the sixth day after delivery a heel prick blood sample is routinely taken for Guthrie testing and a further 0.5 ml blood sample was taken from the same site into a polythene capillary tube (Sarstedt). The blood was allowed to clot, the serum separated by centrifugation and stored at -20°C till analysis.

Quantitation of serum IgA concentrations

A preparation of human secretory IgA purified by the method of Newcomb (9) was kindly provided by Mr B. H. Samson. This preparation was used for radioiodination and as a standard for gel filtration studies. The serum IgA standard used (Hyland References Serum VI Lot 8549N005A) was standardised against the WHO International Standard. The antiserum against serum IgA was raised in rabbits and was kindly provided by Dr T. A. E. Platts Mills (11).

Radioiodination of secretory IgA

Iodine 125 labelled secretory IgA was prepared by the Chloramine T method (4). Forty μg of purified secretory IgA was added to 250 μCi of ^{125}I in the presence of 50 μg Chloramine T. The reaction was terminated after 5 sec by the addition of 170 μg sodium metabisulphite. The radioiodinated protein was separated from inorganic ^{125}I by gel filtration on Sephadex G 50 (Pharmacia) the ^{125}I IgA was further purified by Sepharose 4B (Pharmacia) gel filtration.

Radioimmunoassay of IgA

Sodium phosphate buffer (0.05 mol/l) at pH 7.4 containing 2% horse serum (to reduce non specific binding) was used in making all dilutions and as the buffer and eluting medium in gel filtration and radioimmunoassay. Serum IgA was measured by a double antibody radioimmunoassay. 0.2 ml of a dilution of serum or of the serum IgA standard was added to 0.1 ml of anti IgA at 1/80 000 dilution and incubated at room temperature. Three hours later 1.0 ng of ^{125}I IgA in 0.1 ml of buffer was added and the mixture incubated for a further 3 hours at room temperature. Donkey anti rabbit serum (0.05 ml Wellcome) and non immune rabbit serum (0.05 ml Wellcome) were then added and the mixture incubated 16 hours at 4°C following which bound and free IgA was separated by centrifugation. The antibody bound ^{125}I IgA in the precipitate was measured in an automatic γ -counter (LKB). A standard curve was constructed for each assay by plotting the log of the percent standard counts bound vs log ng of IgA added to the reaction mixture. The IgA concentration of unknown serum samples was calculated from the standard inhibition curve. The sensitivity of the radioimmunoassay procedure as defined by the concentration of IgA which produced a reduction of 10% in the specific binding of ^{125}I IgA tracer alone was 0.4 $\mu\text{g/l}$.

Gel filtration

Gel filtration was carried out using Sepharose 4B (Pharmacia) in a 22x900 mm column with downward flow of eluate. Three ml fractions were collected and stored at -20°C . Immunoassay was performed on the unconcentrated fractions.

Statistical methods

Student's *t* test was used to compare the arithmetic means of serum IgA concentrations in the breast fed and artificially fed neonates. A paired Student's *t* test was used to compare cord blood samples with further serum samples taken from the same baby 6 days later.

Reproducibility of the IgA assay

The intra assay coefficient of variation of the IgA RIA from 8 representative assays was 8.0% ($n=56$). The inter assay coefficient of variation from 9 representative assays was 8.1%.

RESULTS

1. Standard inhibition curve for IgA

The binding of ^{125}I secretory IgA to rabbit anti IgA was displaced in a parallel manner by the Hyland serum IgA standard, two samples of cord blood, one sample of heel prick blood from a 6 day old neonate that had been artificially fed and one sample of heel prick blood from a 6-day old neonate that had been breast fed (Fig. 1). This indicated that immunoassayable IgA was present in cord blood and day 6 heel prick blood.

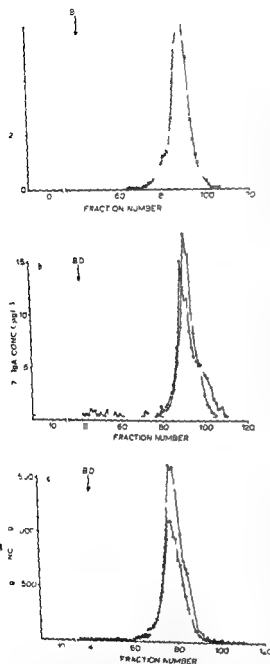


Fig. 2 Gel filtration on Sepharose 4B columns (7×90 cm) of (a) normal adult serum (x-x) (b) cord blood (O-O) pooled heel prick serum from 8 six-day-old neonates (4 artificially fed, 4 breast fed) (●-●) and (c) colostrum (●-●) and milk (O-O). 3 ml fractions were collected and assayed by RIA for IgA using either the serum standard (serum samples) or the secretory standard (colostrum and milk). The arrow indicates the elution volume of a Blue Dextran marker (void volume). The broken line in (b) indicates the limit of detection in this

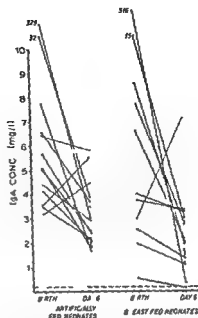


Fig. 3 Serum IgA concentration in the cord blood of 24 newborn infants and heel prick sera of 23 exclusively artificially fed and 25 exclusively breast fed six-day-old neonates. The solid lines indicate cord blood and heel prick sera that were collected from the same baby and the broken line indicates the limit of detection for serum IgA in this particular assay

2 Characterisation of IgA in cord blood and day 6 serum

A single peak of immunoassayable IgA on Sepharose 4B gel filtration was found in cord blood pooled sera from 4 artificially fed and 4 breast fed six day old neonates human colostrum human milk and from adult serum (Fig. 2). Cord blood and heel prick serum IgA eluted at the same volume as adult serum IgA and at a different volume from secretory IgA present in milk and colostrum indicating that the predominant form of IgA in both cord blood and day 6 sera is the 7S monomeric form. The trace of immunoassayable maternal found in infants' serum at the elution volume of secretory IgA was at most 2.5% of the 7S peak suggesting that there is very little 11S IgA in day 6 serum. The 7S IgA standard (Hyland) was therefore used for all subsequent measurements of IgA in cord blood and serum.

3 IgA concentration in cord blood

The concentrations of IgA in 24 cord blood samples ranged from 0.52–516.0 mg/l. Four neonates had cord blood levels that were greater than 10 mg/l and in two of these neonates IgA levels decreased from 516 mg/l to 0.33 mg/l and from 329 mg/l to 3.08 mg/l in 6 days (Fig. 3).

A significant decrease in IgA concentrations occurred during the first 6 days of life in 20 neonates from whom both cord and day 6 sera were tested: this was found in both artificially and breast fed neonates (paired *t* test $t=3.09$, $p<0.01$, Fig. 3). The four neonates with cord blood levels greater than 10 mg/l were excluded from statistical analysis because their cord sera were probably contaminated with maternal blood.

4 IgA concentrations in day 6 serum

Serum IgA levels in 23 exclusively artificially fed and 25 exclusively breast fed babies on the sixth day of life were 2.71 ± 0.25 mg/l and 2.58 ± 0.33 mg/l (arithmetic mean \pm S.E.) respectively (Fig. 3). There was no significant difference between mean IgA concentrations in the two groups showing that exclusive breast feeding had no detectable influence on serum IgA levels after 6 days. One baby had less than 0.14 mg/l of IgA in its serum suggesting that it might be deficient in IgA. This baby also had the lowest IgA concentration in its cord blood.

DISCUSSION

We have shown on the basis of behaviour on gel filtration that IgA in the serum exists in a similar molecular form in both neonates and adults. However IgA concentrations in the neonate are a thousand fold less than those in the adult. In addition we have studied the influence of breast feeding on serum IgA concentrations and have failed to demonstrate an effect.

The evidence for absorption of immuno-

globulins from the neonatal gut is conflicting. Amm and Stiehm (1) found no difference in immune globulin levels between artificially fed and breast fed infants after 4 days of breast feeding. In contrast Izengar & Selye reported significantly higher immunoglobulins on the fifth day of life in colostrum fed infants compared with artificially fed infants (6). Ogra et al. reported a rise in serum IgA levels in 3 infants who were fed colostrum 18 to 74 hours after birth suggesting that the gut is capable of absorbing IgA at this stage (10). In these studies IgA levels were determined by the relatively insensitive technique of single radial immunodiffusion (1, 6) which in our experience has a lower limit of detection of 10 mg/l. In addition Ogra et al. used IgA poliovirus antibody as a marker for IgA in using the technique of radioimmunodiffusion (10). We have used a sensitive radioimmunoassay and low concentrations of IgA were found to be present in all neonates studied. These levels were not influenced by breast feeding. Furthermore we have shown that in infants sera less than 2.5% of the immunoreactive IgA like material behaves like 11S IgA on gel chromatography. Breast feeding would have been expected to produce an immunoreactive peak at the 11S position in neonatal sera if the 11S molecule was absorbed in the intact form by the neonatal gut.

Our data demonstrates firstly that IgA absorption had ceased by the time of sampling at the sixth day and secondly that if there was a period of IgA absorption before the sixth day IgA entering the circulation must have been cleared with a half life value considerably less than the adult serum IgA half life value of approximately 5 days (16). We delayed collection of blood till the sixth day post partum because it was considered unethical to obtain samples earlier as no routine samples were being taken at that time.

Our values for cord blood concentrations of IgA are consistent with the concentrations found by other workers using techniques of similar sensitivity (3, 13). However we found

4 neonates with cord blood concentrations that were greater than 10 mg/l. When these 4 neonates were studied 6 days later their respective serum IgA levels had decreased much more than would be expected if the half life of serum IgA in the infant is similar to that in the adult. We therefore feel that these high values may be due to contamination of cord blood with maternal blood at the time of collection. Earlier reports of raised IgA in cord blood serum (14) may therefore have been due to contamination of cord blood by maternal blood (with thousand fold higher IgA concentrations) at the time of collection.

In the remaining 20 neonates in whom paired cord and day 6 sera were collected a significant decrease in serum IgA was observed in the first 6 days of life. It is possible that this may have been due to transplacental transfer of maternal IgA or to minimal contamination with maternal blood at the time of cord blood collection. We were unfortunately unable to distinguish between these two possibilities with the methodology available. It is unlikely that this decrease is due to cord material interfering with a sensitive inhibition test as parallelism in an inhibition curve was observed between IgA in adult serum and IgA in cord blood. In addition only a single peak of immunoreactivity (at the elution volume of adult serum IgA) was found in cord blood on examination by gel filtration. Any substance that interfered with cord blood IgA determinations would have been expected to elute at a different volume on gel filtration to 7S IgA and therefore would have been detected on immunoassay of the gel filtration fractions.

In one neonate we were unable to detect IgA at the assay dilution used indicating a level less than 0.14 mg/l. This neonate also had the lowest cord blood value among all neonates studied. Unfortunately we had insufficient samples for further analysis using more sensitive conditions for IgA detection but it is considered possible that this infant may be selectively deficient in IgA. We are not aware that this condition has previously been de-

tected in a neonate and follow up of this infant will be undertaken.

In conclusion our data show that evidence of absorption of IgA into the circulation can not be detected in breast fed infants using a very sensitive assay. It is likely that the principle physiological role of breast milk IgA in the infant must be sought at the level of the intestinal lumen or mucosal surfaces.

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We are grateful to Dr D. G. D. Barr who helped us to obtain the blood samples to Dr T. A. E. Platt Mills for the gift of antiserum for radioimmunoassay to Mr R. R. Samson for purified secretory IgA and to Dr W. M. Hunter for advice about radioimmunoassay. One of us (P. L. Y.) was supported by an M.R.C. Clinical Training Fellowship during the period of the study.

REFERENCES

1. Ammann A. J. & Stuehm E. R. Immune globulin levels in colostrum and breast milk and serum from formula and breast fed newborns. *Proc. Soc. Exp. Biol. Med.* 172 1098 1966.
2. Brown W. R., Newcomb R. W. & Ishizaka K. Proteolytic degradation of exocrine and serum immunoglobulin. *J. Clin. Invest.* 49 1174 1970.
3. Faulkner W. & Borella L. Measurement of IgA levels in human cord serum by a new radioimmunoassay. *J. Immunol.* 105 786 1970.
4. Hunter W. M. & Greenwood F. C. Preparation of iodine 131 labelled human growth hormone of high specific activity. *Nature (London)* 194 493 1962.
5. Hurlimann J., Waldesbuhl M. & Zuber C. Human salivary immunoglobulin A. Some immunological and physicochemical characteristics. *Biochim. Biophys. Acta* 181 393 1969.
6. Iyengar L. & Selvaraj R. J. Intestinal absorption of immunoglobulins by newborn infants. *Arch. Dis Child* 47 411 1972.
7. Kenny J. E., Boesman M. I. & Michaels J. G. Bacterial and viral coproantibodies in breast fed infants. *Pediatrics* 34 707 1967.
8. McClelland D. B. L., McGrath J. & Samson R. R. Antimicrobial factors in human milk. *Acta Paediatr. Scand* 67 Suppl. 271 1978.
9. Newcomb R. W., Normansell D. & Stanworth D. R. A structural study of human exocrine IgA globulin. *J. Immunol.* 101 905 1968.
10. Ogra S. S., Weingraub D. & Ogra F. L. Immunologic aspects of human colostrum and milk. *J. Immunol.* 119 745 1977.
11. Platt Mills T. A. E. & Ishizaka K. IgG and IgA diphtheria antitoxin response from human tonsil lymphocytes. *J. Immunol.* 114 1058 1975.
12. Reddy V., Bhaskaram C., Raghuramulu N. & Jaga

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HYPERVISCOSITY OF THE BLOOD AND HAEMOSTASIS IN THE NEWBORN INFANT

P. HENRIKSSON

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ABSTRACT Henriksson P (Department of Paediatrics and Coagulation Laboratory, University of Lund, Allmänna Sjukhuset, Malmö, Sweden). Hyperviscosity of the blood and haemostasis in the newborn infant. *Acta Paediatr Scand* 68: 701-704, 1979. — 15 newborn infants with the hyperviscosity syndrome due to polycythaemia (i.e. a central haematocrit of at least 65% and a raised whole blood viscosity) were examined for changes in their coagulation and fibrinolytic systems. 5 were thrombocytopenic but showed no other signs of activated coagulation. Neither did the only patient with positive ethanol gelation test measuring circulating fibrin monomers. Minute amounts of fibrin/fibrinogen degradation products (FDP) appeared in only two and with only one exception an assay for fibrinolytic activity in plasma was negative. No defects were found in the coagulation system. Thus, in most of the patients there was no demonstrable abnormal proteolysis in the circulation. However, in such infants the normally low levels of antithrombin III (heparin cofactor activity) in combination with the impairment of the microcirculation might increase the risk of thrombotic complications. Haemodilution, preferably with plasma, is therefore advocated in the asymptomatic patients.

KEY WORDS Polycythaemia, hyperviscosity, haemostasis, newborn.

Polycythaemia or hyperviscosity of the blood in the newborn accounts for a variety of symptoms of cardiorespiratory (5-9), cerebral (2-37), metabolic (15) and renal (1) nature. Sporadic cases have been seen where hyperviscosity has been associated with neonatal complications such as necrotizing enterocolitis (23), gangrene of a limb (31), thyrotoxicosis (4) and hydranencephaly (21). For review see Kontras (22) and Gross et al. (11).

Coagulation changes associated with a high haematocrit have been described in 3 cases by Rivers (32). It would, however, appear that no other systematic studies of the haemostatic mechanism in such patients are available. This paper concerns a detailed investigation of the coagulation and fibrinolytic systems in a group of 15 newborns with a central haematocrit of at least 65% and an increased viscosity of whole blood.

CLINICAL MATERIAL

The material consisted of 15 newborns (7 boys and 8 girls) with a central haematocrit ranging from 65 to 73%

(mean 68.4%). Their gestational age ranged from 35 to 42 weeks (mean 40.3). The lowest birthweight was 2250 g and the highest 5000 g (mean 3055 g). Placenta weight ranged from 290 to 760 g (mean 471 g). All the infants were plethoric. Four were small for gestational age. 5 had symptomatic hypoglycaemia. 6 had respiratory symptoms, one (the only preterm infant) had hyperbilirubinaemia and was treated with exchange transfusion at 33 h of age. One had trisomy 21.

Blood sampling. Blood was collected through an umbilical vein catheter inserted for the purpose of haemodilution. The blood sample was diluted in 1/10 volume of 3.8% citrate and plasma was treated as previously described (7,8) and stored at -70°C until examined. The values were corrected for the actual haematocrit.

METHODS

The platelets, fibrinogen, P&P (prothrombin, factor VII and X), factor V, factor VIII, C were determined as described by Nilsson et al. (27) and factor VIII:Ag according to Holmberg & Nilsson (19). Fibrinolytic activity was measured on unheated fibrin plates as described by Nilsson & Olow (7,9). Plasminogen was determined immunochemically according to Ganrot & Niléhn (8) and fibrin/fibrinogen degradation products (FDP) were determined immunochemically according to Niléhn (26) on serum obtained from blood collected in tubes containing thrombin and EACA. The ethanol gelation test of Godal et al. (10) was used as a test for fibrin monomers. Anti-

- deesan V. Antimicrobial factors in human milk. *Acta Paediatr Scand* 64: 709, 1977.
- 13 Schuurman H. J., Hogenaar A. M. & Zegers H. J. M. Cord serum levels of IgM, IgA and Alpha fetoprotein and their inter relationships. *Clin Chim Acta* 79: 363, 1977.
- 14 Stiehm E. H., Ammann A. J. & Cherry J. B. Elevated cord macroglobulins in the diagnosis of intrauterine infections. *N Engl J Med* 275: 971, 1966.
- 15 Tomasi T. B. & Grey H. M. Structure and function of immunoglobulin A. *Proc Allergy* 16: 81, 1972.
- 16 Waldmann T. A. & Strober W. Metabolism of immunoglobulins. *Prog Allergy* 13: 1, 1969.

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nected with high viscosity of the blood in the newborn can be ascribed to impairment of the flow of blood through small vessels particularly post capillary venules (25). Symptoms produced by the primary or parallel condition will add to this.

Rivers (32) is the only author who has demonstrated and characterized coagulation changes in connection with a high haematocrit in the newborn. Three infants with thrombocytopenia and a haematocrit above 65% were studied. Using the serial protamine sulphate test (SDPST) as described by Gurewich & Hutchinson (13) he found circulating fibrin monomers indicating thrombin activity. However, no FDP were found. This absence of co-existing FDP suggested low grade disseminated intravascular coagulation.

In all but one of the present patients the ethanol gelation test was negative i.e. it did not reveal any fibrin monomers. In a comparative study the ethanol gelation test proved equally sensitive as the SDPST while its specificity was debatable (14). Fibrin deposition within the vessels results in secondary fibrinolysis and FDP but practically no FDP were found. Nor could we detect any decrease in those factors normally consumed in patients with intravascular coagulation namely fibrinogen, prothrombin and to some extent factor VIII and AT III. AT III was low in comparison with that in adults but at the same level as in healthy newborns (33) (Fig. 3). The platelet counts were low in 5 but in none of them was the coagulation pattern consistent with activated coagulation. Other possible explanations for the thrombocytopenia in these patients are impaired production due to tissue hypoxia (12) or to a secondary inhibition owing to a predominance of fetal erythropoiesis particularly during intrauterine growth retardation i.e. in infants small for gestational age. The sluggish circulation also in the spleen might possibly increase the disappearance of platelets owing to their prolonged contact with the macrophages. Furthermore in these patients with a small plasma volume in relation

to the raised erythrocyte volume the platelet counts of whole blood will appear low while the concentration of platelets in plasma is normal.

High levels of VIII C and VIII R Ag are often seen in reactive processes and a discrepancy between them i.e. high VIII R Ag in relation to VIII C may indicate pathological proteolysis in the circulation (17). VIII C and VIII R Ag were slightly increased in our patients. Only one showed a high VIII R Ag/VIII C quotient but no other signs of existing coagulation or abnormal fibrinolysis.

In conclusion we could not confirm that newborns with polycythaemia and hyperviscous blood have disturbances suggesting abnormal proteolysis in the blood. However their physiologically low AT III levels (heparin cofactor activity) combined with a sluggish microcirculation are factors which might increase the risk of thrombotic complications impairing the tissue oxygenation still more.

Haemodilution by venesection and replacement infusion preferably with plasma is indicated at least in the symptomatic patients (1, 24, 30).

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REFERENCES

1. Aperia A, Bergqvist G, Broberger O, Thodenius K & Zetterstrom R. Renal function in newborn infants with high hematocrit values before and after isovolumetric haemodilution. *Acta Paediatr Scand* 63: 878, 1974.
2. Baum R S. Hyperviscous blood and perinatal pathology. *Pediatr Res* 1: 88, 1967.
3. Becker A H & Glass H. Twin-to-twin transfusion syndrome. *Am J Dis Child* 106: 6-4, 1963.
4. Bussman Y L, Tillman M L & Pagliara A S. Neonatal thyrotoxicosis associated with the hyperviscosity syndrome. *J Pediatr* 90: 266, 1977.
5. Danks D M & Stevens L H. Neonatal respiratory distress associated with a high haematocrit reading. *Lancet* II: 499, 1964.
6. Ekelund H, Hedner U & Nilsson I M. Fibrinolysis in newborns. *Acta Paediatr Scand* 59: 33, 1970.

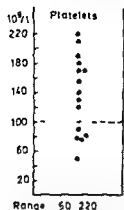


Fig. 1 Platelet counts. Dashed line denotes lower normal limit

thrombin III (AT III) was determined as described by Hedner & Nilsson (16) and α_2 macroglobulin (α_2 M) according to Garrot (7). The whole blood viscosity was measured at 37°C with a Wells Brookfield cone plate microviscometer (36). The hematocrit was measured in duplicate with a micro method.

RESULTS

The results of the individual tests are given in the Figs 1-3. The ethanol gelation test was positive in 1 out of 13 patients examined. FDP appeared in very low concentration in 2 out of 15. Fibrinolysis on fibrin plates was increased in 1 patient of 13 examined. Only 5 cases had a moderate thrombocytopenia and in none of them were the levels of the coagulation factors low. The whole blood viscosity was very high especially at slow shear rates (Fig. 4).

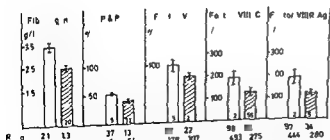


Fig. 2 Mean standard error of the mean and range of fibrinogen, P&P (prothrombin factor VII and factor X), factor V, factor VIII C and factor VIII Ag. Blank columns denote patients, hatched columns healthy newborns according to Holmberg et al. (18). Figures within the columns denote number of infants studied.

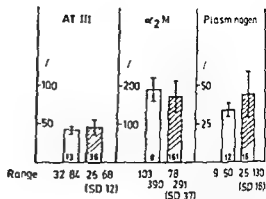


Fig. 3 Mean standard error of the mean and range of antithrombin III, α_2 macroglobulin and plasminogen. Blank columns denote patients, hatched columns healthy newborns according to Teger Nilsson (33) and Ekelund et al. (6). Figures within the columns denote the number of infants studied.

DISCUSSION

In the absence of maternal foetal or twin-to-twin transfusion (3) or as a result of abnormal haemoglobin (34) the etiology of polycythemia in the newborn is obscure. It is often associated with small for gestational age infants (20) or with syndromes such as trisomy 21 (35). The most heterogeneous symptoms con-

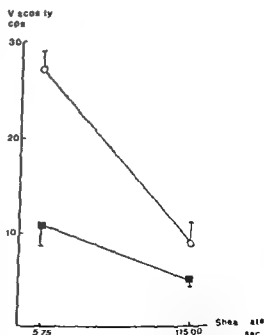


Fig. 4 Mean and standard deviation of whole blood viscosity in eight patients (O) and in 12 healthy adults (■) at shear rates of 115 sec⁻¹ and 5.75 sec⁻¹ respectively.

MEDICINAL IRON TO LOW BIRTH WEIGHT INFANTS

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ABSTRACT Jansson L, Holmberg L and Ekman R (Department of Paediatrics, University of Lund, Malmö General Hospital, Malmö, Sweden and Department of Clinical Chemistry, University Hospital Lund, Sweden). Medicinal iron in low birth weight infants. *Acta Paediatr Scand* 68 705-1979. — Serum ferritin concentrations were measured during the first 6 months of life in 28 low birth weight infants (mean birth weight 1840 g, range 900-2460, mean gestational age 34 weeks, range 29-37) fed a standard formula fortified with ferrous sulphate. Fifteen of the infants received supplementary medicinal iron (ferrous succinate) from 3 weeks of age and 13 only from 2 months of age. All were given vitamin E from 10 days of age. The serum ferritin values did not differ between the groups at 1-2 days, 8-10 weeks or at 6 months. Furthermore, there were no signs of hyperhaemolysis in 8-10 weeks in the group receiving medicinal iron early. The data indicate that the iron content in the formula is sufficient until 2 months of age, but also that there is no disadvantage in starting medicinal iron at 3 weeks of age, if the diet is sufficient in vitamin E.

KEY WORDS S-ferritin, low birth weight, medicinal iron, vitamin E.

The foetus accumulates iron at a rate proportional to the increase in body weight (6). Consequently the low birth weight (LBW) infant is at risk of developing iron deficiency and iron supplementation is recommended (2). The iron can be given either as a formula fortification or as a medicinal iron preparation. The infant formulas produced in Sweden are fortified with either a ferric or a ferrous salt. Because of the poor iron absorption from the ferric salts (11) it has hitherto been the rule to give a medicinal iron preparation (ferrous sulphate or ferrous succinate) in addition to the iron from the formula.

Iron supplementation to LBW infants is, however, not without risks. Because of relative vitamin E deficiency in these infants (9) iron supplementation may increase red cell haemolysis and thus aggravate the physiological anaemia. The purpose of this study was two-fold. First to determine the haematological effects of medicinal iron supplementation in LBW infants fed a standard formula fortified with ferrous sulphate and secondly to assess

the relationship between iron intake and the iron stores in LBW infants during the first 6 months of age. The iron stores of the infants were estimated with the serum ferritin assay which under basal conditions has proved to be a simple and quantitative method for determining iron stores (3, 4, 7, 13, 16).

PATIENTS AND METHODS

The material consisted of 31 LBW infants with a birth weight (BW) ≤ 1000 g and/or a gestational age of ≤ 35 weeks. All infants were born in Malmö General Hospital between December 1975 and May 1977. Infants with haemoglobin values below 140 g/l or above 160 g/l were not accepted, neither were those with signs of haemolysis or those requiring blood transfusions. Two infants with mild respiratory distress were accepted. Three infants were subsequently excluded because of a different feeding regimen. Of the remaining 28 infants, none showed any sign of hepatic or renal disease. The gestational age of each infant was assessed from a combination of maternal data, external characteristics (15) and neurological evaluation (1). The mean birth weight was 1820 g (range 900-2460) and the mean gestational age was 34 weeks (range 29-37).

The infants were randomly distributed among two therapeutic regimens.

- 7 Ganrot P O Determination of α_2 macroglobulin as trypsinprotein esterase *Clin Chim Acta* 14 493 1966
- 8 Ganrot P O & Niléhn J E Immunochemical determination of human plasminogen *Clin Chim Acta* 27 335 1968
- 9 Gatti R A Muster A J Cole R B & Paul M H Neonatal polycythemia with transient cyanosis and cardiorespiratory abnormalities *J Pediatr* 69 1063 1966
- 10 Godal H C & Abildgaard U Gelation of soluble fibrin in plasma by ethanol *Scand J Haematol* 3 342 1966
- 11 Gross G P Hathaway W E & McGaughey H R Hyperviscosity in the neonate *J Pediatr* 87 1004 1973
- 12 Gross S Keefe V & Liebman J The platelets in cyanotic congenital heart disease *Pediatrics* 42 651 1968
- 13 Gurewich V & Hutchinson F Detection of intravascular coagulation by a serial dilution protamine sulfate test *Ann Intern Med* 75 895 1971
- 14 Gurewich V Lipinski B & Lipinski I A comparative study of precipitation by protamine sulfate and ethanol gelation tests *Thromb Res* 2 539 1973
- 15 Haworth J C Dilling L & Younoszai M K Relation of blood glucose to hematocrit birthweight and other body measurements in normal and growth retarded newborn infants *Lancet* II 907 1967
- 16 Hedner U & Nilsson I M Antithrombin III in a clinical material *Thromb Res* 3 631 1973
- 17 Henriksson P & Holmberg L Factor VIII activity and antigen in sick newborns with pathological proteolysis in blood *Acta Paediatr Scand* 67 83 1978
- 18 Holmberg L Henriksson P Ekelund H & Åstedt B Coagulation in the human fetus *J Pediatr* 85 860 1974
- 19 Holmberg L & Nilsson I M Immunologic studies in haemophilia A *Scand J Haematol* 10 12 1973
- 20 Humbert J R Abelson H Hathaway W E & Battaglia F C Polycythemia in small for gestational age infants *J Pediatr* 75 812 1969
- 21 Koffler H Keenan W J & Sutherland J M Hydronecephaly following elevated hematocrit values in a newly born infant *Pediatrics* 54 770 1974
- 22 Kontras S Polycythemia and hyperviscosity syndromes in infants and children *J Pediatr Clin North Am* 19 919 1972
- 23 Leake R D Thanopoulos B & Nieberg R Hyperviscosity syndrome associated with necrotizing enterocolitis *Am J Dis Child* 129 1197 1975
- 24 Mackintosh T F & Walker C H M Blood viscosity in the newborn *Arch Dis Child* 48 547 1973
- 25 Messmer K Hemodilution *Surg Clin North Am* 55 659 1975
- 26 Niléhn J E Separation and estimation of split products of fibrinogen and fibrin in human serum *Thromb Diath Haemorrh* 18 487 1967
- 27 Nilsson I M Blombäck M & Ramgren O Haemophilia in Sweden I Coagulation studies *Acta Med Scand* 170 665 1961
- 28 Nilsson I M Blombäck M & von Francken I On an inherited autosomal haemorrhagic diathesis with antihæmophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* 159 35 1957
- 29 Nilsson I M & Olow B Determination of fibrinogen and fibrinogenolytic activity *Thromb Diath Haemorrh* 8 297 1962
- 30 Oski F A & Naiman J L Hematologic problems in the newborn W B Saunders & Co Philadelphia 1972 pp 78-82
- 31 Papageorgiou A & Stern L Polycythemia and gangrene of an extremity in a newborn infant *J Pediatr* 81 985 1972
- 32 Rivers R P A Coagulation changes associated with a high haematocrit in the newborn infant *Acta Paediatr Scand* 64 449 1975
- 33 Teger Nilsson A C Antithrombin in infancy and childhood *Acta Paediatr Scand* 64 624 1975
- 34 Weatherall D J Polycythemia resulting from abnormal hemoglobin *N Engl J Med* 280 604 1969
- 35 Weinberger M M & Oleinick A Congenital marrow dysfunction in Down's syndrome *J Pediatr* 77 273 1970
- 36 Wells R E Jr Denton R & Merrill D W Measurement of viscosity of biologic fluids by cone plate viscometer *J Lab Clin Med* 57 646 1961
- 37 Wood J L Plethora in the newborn associated with cyanosis and convulsions *J Pediatr* 64 143 1959

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Table 2 *Haematological status at 8-10 weeks of age*Mean values (\pm 1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes ($\times 10^9$ /l)	Platelets ($\times 10^9$ /l)
A	15	103 (10)	60 (30)	383 (171)
B	13	97 (10)	47 (77)	453 (105)

The haematological data at 8-10 weeks and at 6 months of age are given in Table 2 and 3. The groups did not differ significantly from one another in haemoglobin concentration, reticulocyte and platelet counts.

DISCUSSION

In the present study we have investigated the effects of early versus late medicinal iron supplementation to LBW infants. Owing to a low level of α -tocopherol LBW infants are sensitive to oxidant stress from dietary factors. The most important of such factors are supplemental iron and polyunsaturated fatty acids. Even a moderate dose of iron (2 mg/kg/day) increases red cell haemolysis if the diet is rich in polyunsaturated fat (32.4% of the fat as linoleic acid) (17). The infants examined by us were fed a formula in which linoleic acid constituted 17% of the fat. In addition to the tocopherol content of the formula (8 mg/l) the infants received supplemental tocopherol acetate (16.5 mg/day). This amount gives a satisfactory level of serum α -tocopherol (5). Under these feeding conditions we found no signs of increased haemolysis (low Hb, high reticulocyte count) in the group receiving sup-

plemental medicinal iron already from 3 weeks of age. All the infants in our study were fed a formula fortified with 10 mg iron/l as ferrous sulphate, which corresponds to an iron intake of nearly 2 mg/kg/day. This dose has earlier been found to prevent iron deficiency in LBW infants (4) when the iron is present as an readily available salt. According to Rios et al (11) fortification with ferric salts such as ferric orthophosphate and ferric pyrophosphate is not sufficient to meet the needs of the infant. Nevertheless formulas fortified with ferric salts are still on sale in Sweden.

In the infants studied by us we found no difference in α -ferritin at two months of age, whether the infants had received supplemental medicinal iron from 3 weeks of age or not. However, α -ferritin values may be difficult to evaluate when supplemental iron is given, as iron therapy can by itself raise α -ferritin in iron deficiency before the iron stores are fully saturated (13, 14). The effect of the dose of supplemental iron on α -ferritin has not been fully evaluated, but as α -ferritin showed the same development during the first six months in the two groups there seems to be no advantage with the early medicinal iron supplementation.

When investigating the requirement of iron supplementation to LBW infants, the length of breast feeding should also be considered. In our study only two of the mothers were able to breast feed their infants for 6 weeks. The value of prolonged breast feeding in protecting term infants from iron deficiency has been stressed by Saanen et al (12). In LBW infants, however, Lundström et al (8) found no differences in α -ferritin and Hb at 3 months of age, whether the infants were fed breast milk

Table 3 *Haematological status at 6 months of age*Mean values (\pm 1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes ($\times 10^9$ /l)
A	10	115 (7)	70 (15)
B	9	115 (5)	15 (8)

Table 1 Birth weight and haematological status at birth in the two therapy groups

Mean values (± 1 SD)

Therapy group	No	Birth weight (gram)	Haemoglobin (g/l)	Reticulocytes ($\times 10^9/l$)	Platelets ($\times 10^9/l$)
A	15	1 855 (430)	200 (27)	135 (75)	140 (71)
B	13	1 779 (327)	191 (29)	166 (75)	160 (73)

(A) Ferrous succinate 2-3 mg/kg/day (Ferromyn S* AB Hassle) from 3 weeks of age

(B) Ferrous succinate 2-3 mg/kg/day from 2 months of age

All the infants were given the following vitamin preparations from 10 days of age: 1/2 ml Protovit* (Roche) supplying vitamin A C D the B complex and 1.5 mg tocopherol acetate/day tetrahydrofolic acid 50 μ g/day (Leucovorn* Lederle) and 15 mg tocopherol acetate/day (E vitamin* AB ACO)

Feeding, routines All the infants were given human pasteurized milk for a period of at least 2 weeks or until they weighed 2 100 g. If the breast milk supply by the mothers was then insufficient to satisfy the infants' requirement a commercial standard formula Milkotal (AB Findus) was given. Milkotal contains 8 mg tocopherol acetate/l and linoleic acid accounts for 17% of the total fat. The formula is fortified with 10 mg ferrous sulphate/l. After the discharge from hospital two infants were partly breast fed until 6 weeks of age. Solid food and cereals were introduced at 4-5 months of age.

The infants were examined haematologically within the first 24 hours at 8-10 weeks and at 6 months of age. Blood for determining the serum ferritin concentration was taken at 24-48 hours at 8-10 weeks and at 6 months of age. All blood samples were obtained by heel puncture. Sera were stored at -70°C until analysed.

Serum ferritin was determined according to the method of Miles *et al.* (10) with reagents from Ramco Laboratories Inc. Houston Texas. The statistical analyses were calculated with Student's *t* test. In the case of serum ferritin the statistical analyses were performed after log transformation (3 4 7 13 16).

RESULTS

The mean birth weights and the haematological data at birth in the groups are given in Table 1. The two groups were similar in birth weight, haemoglobin concentration, reticulocyte and platelet counts.

Fig. 1 shows the serum ferritin concentration in the two groups at 1-2 days, 8-10 weeks and at 6 months of age. In group A (medicinal iron from 3 weeks) the mean serum ferritin fell from 102 μ g/l (range 34-220) at 1-2 days to 56 μ g/l

(range 16-175) at 8-10 weeks and to 26 μ g/l (range 18-45) at 6 months of age. In group B (medicinal iron from 2 months) we found a similar fall in serum ferritin from 100 μ g/l (range 45-200) at 1-2 days to 72 μ g/l (range 21-170) at 8-10 weeks of age to 28 μ g/l (range 10-115) at 6 months of age. Serum ferritin did not differ between the groups on any of these occasions. This was also true when only those of the infants with a birth weight of ≤ 2 000 g were taken into account. In these infants the mean serum ferritin at 8-10 weeks in group A (11 infants) was 27 (range 16-116) μ g/l and in group B (11 infants) 47 (range 21-170) μ g/l.

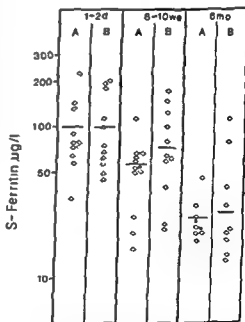


Fig. 1 Serum ferritin concentration in 2 groups of LBW infants at 1-2 days, 8-10 weeks and at 6 months of age. (A) Ferrous succinate from 3 weeks. (B) Ferrous succinate from 2 months. The bars in the figure denote geometrical means. \square infants with BW ≤ 2 000 g. \bullet infants with BW > 2 000 g.

Table 2 *Haematological status at 8-10 weeks of age*Mean values (± 1 S D)

Therapy group	Nm	Haemoglobin (g/l)	Reticulocytes ($\times 10^9/l$)	Platelets ($\times 10^9/l$)
A	15	103 (10)	60 (30)	383 (171)
B	13	97 (10)	47 (7)	453 (105)

The haematological data at 8-10 weeks and at 6 months of age are given in Table 2 and 3. The groups did not differ significantly from one another in haemoglobin concentration, reticulocyte and platelet counts.

DISCUSSION

In the present study we have investigated the effects of early versus late medicinal iron supplementation to LBW infants. Owing to a low level of α -tocopherol LBW infants are sensitive to oxidant stress from dietary factors. The most important of such factors are supplemental iron and polyunsaturated fatty acids. Even a moderate dose of iron (2 mg/kg/day) increases red cell haemolysis if the diet is rich in polyunsaturated fat (32.4% of the fat as linoleic acid) (17). The infants examined by us were fed a formula in which linoleic acid constituted 17% of the fat. In addition to the tocopherol content of the formula (8 mg/l) the infants received supplemental tocopherol acetate (16.5 mg/day). This amount gives a satisfactory level of serum α -tocopherol (5). Under these feeding conditions we found no signs of increased haemolysis (low Hb, high reticulocyte count) in the group receiving sup-

plemental medicinal iron already from 3 weeks of age. All the infants in our study were fed a formula fortified with 10 mg iron/l as ferrous sulphate, which corresponds to an iron intake of nearly 2 mg/kg/day. This dose has earlier been found to prevent iron deficiency in LBW infants (4), i.e. when the iron is present as an readily available salt. According to Rios et al (11) fortification with ferric salts such as ferric orthophosphate and ferric pyrophosphate is not sufficient to meet the needs of the infant. Nevertheless formulas fortified with ferric salts are still on sale in Sweden.

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When investigating the requirement of iron supplementation to LBW infants, the length of breast feeding should also be considered. In our study only two of the mothers were able to breast feed their infants for 6 weeks. The value of prolonged breast feeding in protecting term infants from iron deficiency has been stressed by Saarinen et al (12). In LBW infants, however, Lundstrom et al (8) found no differences in α -ferritin and Hb at 3 months of age, whether the infants were fed breast milk

Table 3 *Haematological status at 6 months of age*Mean values (± 1 S D)

Therapy group	No	Haemoglobin (g/l)	Reticulocytes ($\times 10^9/l$)
A	10	115 (7)	0 (15)
B	9	115 (5)	15 (8)

or a formula not fortified with iron. Their findings suggest that iron supplement should be given also to breast fed LBW infants.

REFERENCES

1 Amiel-Tisson C. Neurological evaluation of the maturity of newborn infants. *Arch Dis Child* 43: 89 1968.

2 Committee on Nutrition. Iron supplementation for infants. *Pediatrics* 59: 765 1976.

3 Cook C D, Lipschitz D A, Miles L E M & Finch C A. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 27: 681 1974.

4 Jacobs A, Mitter F, Worwood M, Beumish M R & Wardrop C A. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br Med J* 14: 206 1972.

5 Jansson L, Holmberg L, Nilsson B & Johansson B. Vitamin E requirements of preterm infants. *Acta Paediatr Scand* 67: 459 1978.

6 Lanzkovsky O. Iron metabolism in the newborn infant. *Clin Endocrinol Metabol* 5: 149 1976.

7 Lipschitz D A, Cook J D & Finch C A. A clinical evaluation of serum ferritin as an index of iron stores. *N Engl J Med* 209: 1213 1974.

8 Lundstrom U, Simes M A & Dallman P R. At what age does iron supplementation become necessary in low birth weight infants? *J Pediatr* 91: 878 1977.

9 Melhorn D K & Gross S. Vitamin E dependent anaemia in the premature infant. I. Effects of large doses of medicinal iron. *J Pediatr* 79: 560 1971.

10 Miles L E M, Lipschitz D A, Bieber C P & Cook J D. Measurement of serum ferritin by a 2 site

immunoradiometric assay. *Anal Biochem* 61: 69 1974.

11 Rios E, Hunter R E, Cook J D, Smith N J & Finch C A. The absorption of iron as supplement in infant cereal and infant formulas. *Pediatrics* 59: 686 1975.

12 Saarnen U M, Simes M A & Dallman P R. Iron absorption in infants. High bioavailability of breast milk iron is indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. *J Pediatr* 91: 36 1977.

13 Simes M A, Addiego J E & Dallman P R. Ferritin in serum. Diagnosis of iron deficiency and iron overload in infants and children. *Blood* 41: 581 1974.

14 Thomas W J, Koenig H M, Lightsey A L Jr & Green R. Free erythrocyte porphyrin, Hemoglobin ratios, serum ferritin and transferrin saturation levels during treatment of infants with iron deficiency anaemia. *Blood* 49: 455 1977.

15 Usher R, McLean F & Scott K. Judgement of foetal age. II. Clinical significance of gestational age and an objective method for its assessment. *Pediatr Clin North Am* 13: 835 1966.

16 Walters G O, Miller F M & Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 26: 770 1973.

17 Williams M L, Shott R J, O'Neal P L & Oski F A. Role of dietary iron and fat on vitamin E deficiency of infancy. *N Engl J Med* 297: 887 1975.

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THE NUMBER OF POLYMORPHONUCLEAR LEUKOCYTES IN RELATION TO GESTATIONAL AGE IN THE NEWBORN

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ABSTRACT Coulombel L Dehan M Tchernia G Hill C and Vial M (Laboratoire Central d'Hématologie and Service de réanimation neonatale Hopital Antoine Beclere Clamart France) The number of polymorphonuclear leukocytes in relation to gestational and post natal ages in non infected distressed newborns Acta Paediatr Scand 68 709 1979 — 173 distressed newborn infants without evidence of bacterial infection were investigated at 12 hour intervals for the number of polymorphonuclear leukocytes during the first 5 days of life The results showed a significant difference in the number of polymorphonuclear leukocytes in relation to both gestational and post natal ages The study stresses the necessity of taking into account these differences in interpreting neutropenia as a sign of bacterial infection in neonates

KEY WORDS Newborn infants bacterial infection neutropenia

Within the first days of life the existence of neutropenia is usually considered a sign of serious bacterial infection (1 2 5 6). In an attempt to define numerical limits on the definition of neutropenia we studied in a population of distressed but non infected newborns the variations in the number of polymorphonuclear leukocytes (PMN) in relation both to gestational and post natal ages.

MATERIAL AND METHODS

All newborn infants of age less than 5 days admitted to the neonatal intensive care unit during a nine month period were included in this study. All these infants had some kind of distress and none of them could be considered as normal infants. 70% of all blood samples and 85% of the first 48 hours samples were obtained by umbilical catheter. Other samples were obtained by heel pricks. The variation of hemoglobin values in the different samples drawn at 1 hour intervals did not exceed 15 g/l. Thus we assumed that all WBC counts could be taken in account and compared even when the sampling technique was different. Total leukocyte enumeration was performed by a Coulter S blood cell counter and was verified by hemocytometer if the white blood cell count was less than 3000/mm³. A differential

count was performed by classifying 200 nucleated cells on a smear stained with May Grunwald-Giemsa.

These observations were initiated when the infant entered the unit, were repeated every 12 hours up to 48 hours of age and were continued every 24 hours afterwards through the 4th day of life. 240 newborn infants were included in this investigation. 67 were excluded from the statistical analysis either because bacterial infection was verified (15 infants) or because infection was thought probable but not proven by bacteriological results (52 infants). The remaining 173 infants without bacterial infection were retained for the statistical analysis. They were subdivided into 3 groups of different gestational age. Namely group 1: 37 weeks or more, group 2: 33 to 36 weeks and group 3: 31 weeks or less. Among these 173 newborns samples during the first 12 hours of life were obtained for 132 infants. For the time intervals after the first 12 hours we included data from all available patients even if the early blood samples had not been obtained.

RESULTS

As shown in Fig. 1 within the first twelve hours of life in 132 newborns a correlation appeared between gestational age and the number of PMN/mm³ as gestational age progressed the mean number of PMN/mm³ progressively increased. During this initial 12 hour period the mean ± 2 S.E.M. number of

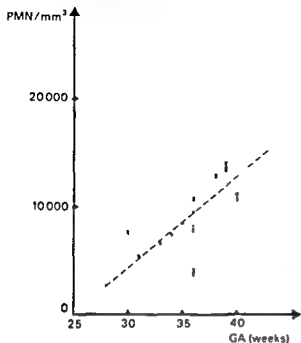


Fig. 1 PMN/mm³ in relation to gestational age in 132 newborns within the first 12 hours of life. The formula for the equation of the regression line allowing calculation of the number of PMN in relation to gestational age is $PMN \times GA - 16700$ $r=0.52$ $p<0.001$.

PMN was $12000 \pm 1400/\text{mm}^3$ in 58 infants of group 1, 8000 ± 1300 in 49 infants of group 2 and $6000 \pm 1000/\text{mm}^3$ in 25 infants of group 3. The evolution of the number of PMN/mm³ is compared in the 3 gestational age groups appears in Fig. 2.

The variation of PMN after birth was similar in the 3 groups of infants. After an initial increase between 12 and 24 hours of life, there was a decrease in all groups that was more pronounced in group 1 than in groups 2 and 3 between the 24th and 72nd hour of life. This greater decrease in group 1 resulted in similar levels of PMN in all 3 groups by the 4th day (Fig. 2).

DISCUSSION

During fetal life, hematopoiesis occurs early in the yolk sac during the third gestational week. Then fetal hematopoietic cells migrate successively to different sites primarily to the fetal liver and by the 4th month to the bone marrow (4). At this stage, there is a major commitment to erythroid differentiation but a proportion of cells have been shown to be

capable of granulocyte differentiation in other environments as shown by Moore & Williams (3). In vivo, this granulocytic differentiation mainly occurs in bone marrow during the last gestational weeks and gradually increases. This process can explain the correlation between the number of PMN and the gestational age. The loss of this correlation during the first week after birth suggests that natal or postnatal events lead to a regulation of the granulocyte population which eliminates the differences in children of various gestational ages by the end of the 1st week of life.

This fact, to our knowledge, had not been found in previous studies. In sick newborn infants, early detection and prompt treatment of bacterial infection is necessary to avoid a fulminant course of disease leading frequently to death. Since neutropenia has been shown to represent a useful and early sign of severe bacterial infections, it appears important to define the limits of this criterion. Our results emphasize the necessity of referring to both gestational and postnatal ages in interpreting the significance of the number of PMN in sick newborns. For example (Fig. 2), 5000 PMN/mm³ at less than 12 hours of age is a normal figure for an infant born at 32 weeks of gestation but is pathologic for a term neonate of the same postnatal age.

At present, these figures are employed routinely in our intensive care unit and are useful in the diagnosis of neonatal infection.

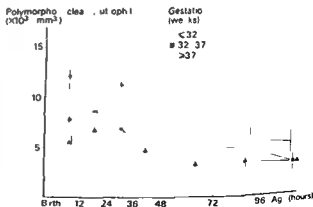


Fig. 2 Evolution of PMN/mm³ as compared in the 3 gestational groups.

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REFERENCES

- 1 Gregori J & Hey E Blood neutrophil response to bacterial infection in the first month of life *Arch Dis Child* 47 747 1972
- 2 Kuchler H Fricker H & Gugler E La formule sanguine dans le diagnostic precoce de la septicemie du nouveau né *Helv Paediatr Acta* 31 33 1976
- 3 Moore M A S & Williams N Analysis of proliferation and differentiation of fetal granulocyte macrophage progenitor cells in haemopoietic tissue *Cell Tissue Kinet* 6 461 1973

- 4 Oski F A & Nauman J L *Hematologic problems in the newborn* W B Saunders Company Philadelphia 1972 2nd ed p 377
- 5 Xanthou M Leucocyte blood picture in ill newborn babies *Arch Dis Child* 47 731 1972
- 6 Zipurski A & Jaber H M The haematology of bacterial infection in newborn infants *Clin Haematol* 7 175 1978

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UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

II Infections in Relation to Catheterization

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ABSTRACT Wessström G and Finnström O (Department of Paediatrics, University Hospital, Linköping, Sweden). Umbilical artery catheterization in newborns. II Infections in relation to catheterization. *Acta Paediatr Scand* 68 713 1979.—Infections as a complication of umbilical artery catheterization were studied in 65 sick newborn infants. Every second day during the catheterization period peripheral blood cultures as well as blood samples for white cell count and platelets were taken. Cultures were taken from the catheter tips and from the umbilicus at the time of withdrawal of the catheter. Local or systemic antibiotics were not used prophylactically but on rather broad clinical indications. No case of septicemia was found but 8 infants had positive blood cultures and 16 had positive catheter tip cultures. Positive catheter tip culture occurred more often in infants who were born before 32 weeks of gestational age. Neither the duration of the catheterization nor the treatment with antibiotics influenced significantly the frequency of positive cultures.

KEY WORDS Newborn infant, umbilical artery catheterization, infection.

Umbilical artery catheterization is an accepted procedure for obtaining arterial acid base values in sick newborn infants. During the almost 20 years in which this method has been used various complications have been described such as thrombosis/embolism, vascular perforation, ischemic necrosis of abdominal viscera, hemorrhage, blanching of the legs and infection. These have been reviewed in an earlier paper (31).

A few prospective studies have evaluated the risk of local or systemic infection associated with umbilical artery catheterization. The frequency of septicemia in these studies varied between 0 and 8% (4, 12, 26, 29) and the frequency of bacterial colonization of the catheters between 6 and 55% (8, 22, 25).

The value of local and/or systemic antibiotics to prevent infections at umbilical catheterization has been investigated with varying results (3, 4, 5, 22, 24, 29).

The present study was undertaken to analyze the following problems in connection with catheterization:

- the risk of systemic infection
- the significance of positive catheter tip and blood cultures
- the value of antibiotics in the prevention of infection
- the correlation between certain clinical parameters and the risk for systemic infection

MATERIAL

During a 19 month period 71 sick newborn infants had an umbilical artery catheter introduced. Six infants were excluded from the study: 3 because of early death before cultures had been taken and 3 for other reasons. The indications for catheterization have been given in an earlier paper (31). Forty-seven infants had a low birth weight (≤ 2500 g), 54 were preterm (≤ 37 weeks of gestational age), 17 had severe postnatal asphyxia (Apgar ≤ 6 at 5 min), in 13 there was an early rupture of the membranes (> 24 hours) and 74 experienced a complicated delivery such as breech presentation or caesarean section on foetal indication.

Fifty-three infants were fed with human milk. In 46 the feeding was started during the first 3 days of life and in 7 after the 3rd day but during the catheterization period. The remaining 12 infants did not receive human milk: 7 because of severe illness or early death and 5 because no human milk was available. The human milk was stored

Table 1 *Clinical data of the 65 infants*

	Mean	S D	Range
Birth weight (g)	2 103	799	960-4 670
Gestational age (weeks)	34.7	3.5	26-43
Apgar score at 5 min	7.6	2.3	1-10
Duration of catheterization (hours)	69.3	40.1	6-173
Degree of illness (score 1-5)	2.5	1.0	1-5

frozen, thawed and rapidly heated to about 95 °C and then cooled in a water bath before use.

Blood cultures were performed in 61 infants, catheter tip cultures in 58 and umbilical cultures in 53. All types of cultures could not be done in all infants.

The mean values and range for birth weight, gestational age, Apgar score at 5 min, duration of catheterization and degree of illness are given in Table 1. Degree of illness was subjectively estimated according to a 5 degree scale where score 5 consisted of the infants who died, score 4 and 3 of severely sick infants as exemplified by the need for mechanical ventilation or continuous positive airway pressure (CPAP) and score 2 and 1 the remaining less sick infants.

METHODS

The catheterization was performed with a strictly aseptic technique. The umbilical stump and the surrounding skin were cleaned with 0.5% Chlorhexidine alcohol. Mainly for the purpose of studying the frequency of thromboses (31) two types of catheters, Argyle umbilical artery and Argyle feeding tube, were used and inserted in the aorta either short (L_{2-3}) or long (T_{4-11}). After insertion of the catheter the umbilicus as well as the stopcock were covered with a sterile dressing. The stopcock and supplying connections were changed daily. There was no topical application of antibiotics. The catheter was used both for blood sampling and for infusion. When drawing blood sterile gloves were worn. A 10% glucose solution was infused in all infants. If necessary blood transfusions or

Table 2 *Indications for treatment with antibiotics in 22 infants*

	No of infants
Intrauterine infection suspected	5
Postnatal septicemia suspected	9
Pneumonia suspected	5
Positive blood culture	2
Necrotizing enterocolitis	1
Total	22

Table 3 *Number of positive cultures and the types of the bacteria*

In 3 instances more than one type of bacteria was found in the culture.

	Blood culture	Catheter tip culture	Umbilical culture
Gram positive bacteria			
Staphylococcus epidermidis	6	1	8
Staphylococcus aureus		5	6
Beta hemolytic streptococcus		4	1
Enterococcus		1	1
Alpha streptococcus			1
Gamma streptococcus			1
Diphtheroids	1		
Gram negative bacteria			
Escherichia coli	1	2	1
Klebsiella/Enterobacter		2	1
Proteus		1	
Pseudomonas		1	
Coliforms	1		
Number of strains cultured	9	17	21
Number of infants with positive cultures	8	16	21

Two group A, 3 non group A or B but not further serotyped.

other fluids, e.g. sodium bicarbonate, were given through the catheter.

Prophylactic antibiotics were not used but the clinical indications for antibiotics were rather broad (Table 2). The physician in charge decided if the infants were to be treated in which case a combination of gentamicin and ampicillin was used, except in two infants.

Every second day during the time the catheter was in situ peripheral blood samples were obtained for anaerobic and aerobic cultures. The samples were drawn by the vacuum system directly into a supplemented peptone broth blood culture medium (Becton Dickinson, Rutherford, USA). At removal the distal part (1-2 cm) of the catheter was cut off and cultured in a 0.1% Dextrose broth. At the same time an umbilical swab was cultured in the same type of broth. The different bacteria were classified according to Cowan & Steel (11). Total white cells and platelets were analysed every second day. Clinical signs and symptoms which could be referred to infection were specially noticed.

RESULTS

The results of the bacteriological examinations are shown in Table 3. Only one infant had definite pathogenic bacteria in his blood culture.

Table 4 Culture results in relation to the degree of illness

Degree of illness (score)	Blood culture			Catheter tip culture		
	No of infants	Positive culture		No of infants	Positive culture	
		n	%		n	%
1-2	37	5	13.5	38	11	28.9
3-4	19	3	15.8	11	5	27.8
5	4	0	0	7	0	0
Total	61	8	13.1	56	16	27.6

(*E. coli*) but together with a presumably non pathogenic organism (*S. epidermidis*). Five of the 8 infants with positive blood cultures later had a negative culture. The cultures of the catheter tips showed presumably pathogenic bacteria in all but one of the 16 positive cases.

In 57 infants both blood and catheter cultures were done. None of them had the same bacteria in both cultures. Cultures from the catheter tip as well as from the umbilicus were done in 52 infants. Five of them had the same type of bacteria in both cultures but in 2 of the umbilical cultures there were 2 different strains.

Cultures from the catheter tip were positive in two of 19 infants (11%) with and in 14 of 39 without antibiotic therapy (36%). Fourteen infants received antibiotic therapy before the last blood culture was taken. One of them had a positive blood culture (7%) compared with 7 positive cultures among the 47 infants without antibiotic therapy (15%). None of these differences were statistically significant however.

The mean duration of the catheterization was almost the same: 76 and 71 hours respectively in infants with and without positive catheter tip culture. Corresponding values for infants with and without positive blood culture were 92 and 65 hours respectively: a non significant difference however.

Positive catheter tip cultures were found more often in side hole than in end hole catheters: 32 and 20% respectively. The difference

was not statistically significant. No difference was noticed between long and short catheters.

The number of positive blood or catheter tip cultures was unrelated to the infants' degree of illness (Table 4).

Infants younger than 32 weeks of gestational age had an increased incidence of positive catheter tip cultures ($p < 0.01$) but all their blood cultures were negative.

Sixteen infants who developed arterial thromboses as described elsewhere (31) did not show an increased number of positive cultures nor did the fourteen infants who received blood transfusions through the catheter.

The infants who received breast milk late or not at all during the catheterization did not show an increased number of positive catheter or blood cultures.

There was no difference with regard to the number of white cells or platelets between infants with and without positive cultures.

None of the infants had clinical signs or symptoms compatible with septicemia in combination with a positive blood culture. Nor had any of the infants who died any signs of bacterial infection at autopsy.

DISCUSSION

Sepsis neonatorum is defined as a disease of infants who are less than 1 month of age, are clinically ill, and who have positive blood cultures (20). The incidence is 1-3 cases per 1000 live born infants (16-32).

Umbilical artery catheterization predisposes to infections (27-29) as does preterm delivery postnatal asphyxia and other adverse perinatal factors such as early rupture of the membranes, complicated deliveries and resuscitation procedures (6, 7, 18-21). All infants in the present study were high risk infants for neonatal septicemia. Besides umbilical artery catheterization they had as a rule one or more predisposing factors.

Previous prospective studies in which the frequency of infections in relation to umbilical artery catheterization has been studied are few. Strauss et al. (26) found sepsis in 2 of 26 infants (8%) and Van Vleet et al. (29) in 11 of 229 (5%) while others did not find any cases of sepsis at all (12-14). Krauss et al. (22) who used arterial catheters of side hole type found positive catheter tip cultures in 6 of 11 infants (55%) while Powers et al. (25) and Carlson et al. (8) who used end hole catheters found positive catheter tip cultures in only 9 and 6% respectively. Carlson et al. suggested that these great differences could be due to the use of different types and positions of the catheter. This explanation can be confirmed to some extent in the present study with a small but not significant difference in the frequency of positive catheter tip cultures between side and end hole catheters. There was no such difference however with regard to the position of the catheter tip.

The duration of the catheterization in relation to infection has previously been studied in connection with umbilical artery, umbilical vein and peripheral vein catheters. Thus several studies have shown a relation between the frequency of infection and the duration of the catheterization with umbilical vein catheters as well as with peripheral vein catheters (9-12). Several authors with one exception (27) have failed to demonstrate such a relation for umbilical artery catheters (4, 22-29). The failure to show this relation in the present and earlier studies may depend on a relatively short mean duration of the catheterization period.

The increased frequency of positive catheter tip cultures in the most preterm infants in the present investigation is in agreement with earlier findings (7, 13-21).

An important question is the time for colonization of the catheters. This might theoretically occur on insertion of the catheter during the time the catheter is in situ or on withdrawal of the catheter. Previous studies have shown that the umbilicus is sterile in most cases during the first day of life but later a rapid colonization takes place (15-19). Once the umbilicus has been colonized umbilical cultures will as a rule remain positive in spite of intense cleaning (2). In the present study all infants had the umbilical artery catheter introduced during the first 12 hours but 9 infants were later recatheterized of whom 3 (33%) had a positive catheter tip culture. Krauss et al. (22) demonstrated bacterial growth inside the lumen of the catheters which speaks against colonization on withdrawal. Colonization on insertion or withdrawal thus seems less likely so does the present finding that in only a few infants did the same bacteria grow from both the umbilicus and the catheter tip.

The importance of a positive catheter tip culture is not clear. The occurrence of bacterial growth on the catheter tip might enhance the risk for developing septicemia. In spite of the high rate of positive catheter tip cultures the risk for septicemia seems to be small as shown by us and by others (4, 12, 22).

The importance of a positive blood culture from a newborn infant is also difficult to evaluate. A positive blood culture might be an indication of contamination, bacteremia or septicemia. Eight infants (13%) had positive blood cultures to be compared with the findings of Albers et al. (1) of 14% in healthy newborn infants. They thought that positive cultures depend either on contamination or low grade self limited transient bacteremia. This interpretation might hold also for our study since 7 of 8 positive blood cultures showed growth of bacteria that often are non pathogenic. In the last culture two types of bacteria

grew out which might be an indication of a contamination

Several investigators have evaluated the effect of antibiotics as prophylaxis against infection in connection with umbilical artery catheterization. In most of these studies as in the present antibiotics did not significantly decrease the frequency of positive blood or catheter tip cultures (5, 12, 22, 29). Bard et al (4) however found in a randomized study a significantly increased number of positive blood and catheter tip cultures in non-treated infants. We agree with those authors including Bard et al (4) who believe that prophylactic antibiotics should not be used for umbilical artery catheterization (5, 29). Prophylactic antibiotics can select antibiotic resistant strains (30) and delay a normal colonization of the intestines with increased risk for fungal infections (19).

The antibacterial effect of breast milk is well known (17) and the low incidence of severe infections in the present study might to some extent depend on the early feeding with human milk even if in the handling of the milk with freezing and heating some of the antimicrobial factors were destroyed (23).

The peripheral white blood cell count is a non-specific test which may be difficult to interpret in newborn infants (7, 18, 32). Xantou (33) showed an increase in segmented and non-segmented polymorphonuclear leucocytes in infected infants but she also found the total white cell count to be of limited value in the diagnosis. However Tollner et al (28) found a significant increase in the total white cell count at the onset of septicemia and a marked drop when the disease advanced. Thrombocytopenia is thought to be a relatively sensitive laboratory sign of septicemia in the neonatal period (10, 18). In the present study there was no increase in leucocytes or decrease in thrombocytes in relation to positive cultures which speaks against the presence of septicemia in these infants.

In conclusion we found that the risk of septicemia during umbilical artery catheteriza-

tion is low if a strict aseptic technique is used in the catheterization and handling of the catheter. The value of arterial catheterization in the control and the treatment of seriously ill newborn infants thus outweighs the possible risk of infection.

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REFERENCES

1. Albers W H, Tyler C W & Boxerbaum B. Asymptomatic bacteremia in the newborn infant. *J Pediatr* 69: 193, 1966.
2. Anagnostakis D, Kamba A, Petrochidou V, Arseni A & Matsamotis N. Risk of infection associated with umbilical vein catheterization. *J Pediatr* 86: 759, 1975.
3. Balagtas R C, Bell C E, Edwards L D & Levin S. Risk of local and systemic infections associated with umbilical vein catheterization. A prospective study in 86 newborn patients. *Pediatrics* 48: 359, 1971.
4. Bard H, Albert G, Teasdale F, Doray B & Martineau B. Prophylactic antibiotics in chronic umbilical artery catheterization in respiratory distress syndrome. *Arch Dis Child* 48: 630, 1973.
5. Bhatt D R, Hodgman J E & Tatter D. Evaluation of prophylactic antibiotics during umbilical catheterization in newborns. *Clin Res* 18: 217, 1970.
6. Blanc W A. Pathways of fetal and early neonatal infection. *J Pediatr* 59: 473, 1961.
7. Buctow K L, Klein S W & Lane R H. Septicemia in premature infants. *Am J Dis Child* 110: 29, 1965.
8. Casalino M B & Lipsitz J J. Contamination of umbilical catheters. *J Pediatr* 78: 1077, 1971.
9. Collins M N, Braun P A, Zinner H & Kass M H. Risk of local and systemic infection with polyethylene intravenous catheters. A prospective study of 213 catheterizations. *N Engl J Med* 279: 340, 1968.
10. Corrigan J J. Thrombocytopenia: A laboratory sign of septicemia in infants and children. *J Pediatr* 85: 219, 1974.
11. Cowan S T & Steel K J. *Manual for the identification of medical bacteria*. New York, 1974. 2nd ed.
12. Daschner F, Adam D & Marget W. Sepsis und Kontamination bei Nabel und Venenkathetern. *Monatssch Kinderheilkd* 172: 49, 1974.
13. Davies P A. Bacterial infection in the fetus and newborn. *Arch Dis Child* 46: 1, 1971.
14. Dorand M D, Cook L M & Andrews H F. Umbilical vessel catheterization. The low incidence of

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REFERENCES

1. Albers W H, Tyler C W & Boxerbaum B. Asymptomatic bacteremia in the newborn infant. *J Pediatr* 69: 193, 1966.
2. Anagnostakis D, Kamba A, Petrochilou V, Arseni A & Matsaniotis N. Risk of infection associated with umbilical vein catheterization. *J Pediatr* 86: 749, 1975.
3. Balagtas R C, Bell C E, Edwards L D & Levin S. Risk of local and systemic infections associated with umbilical vein catheterization. A prospective study in 86 newborn patients. *Pediatrics* 48: 359, 1971.
4. Bard H, Albert G, Teasdale F, Doray B & Martineau B. Prophylactic antibiotics in chronic umbilical artery catheterization in respiratory distress syndrome. *Arch Dis Child* 48: 630, 1973.
5. Bhatt D H, Hodgman J E & Taiter D. Evaluation of prophylactic antibiotics during umbilical catheterization in newborns. *Clin Res* 18: 217, 1970.
6. Blanc W A. Pathways of fetal and early neonatal infection. *J Pediatr* 59: 473, 1961.
7. Buetow J C, Klein S W & Lane R H. Septicemia in premature infants. *Am J Dis Child* 110: 29, 1965.
8. Casalino M B & Lipsitz P J. Contamination of umbilical catheters. *J Pediatr* 78: 1077, 1971.
9. Collins R N, Braun P A, Zinner S H & Kass E H. Risk of local and systemic infection with polyethylene intravenous catheters. A prospective study of 713 catheterizations. *N Engl J Med* 279: 340, 1968.
10. Corrigan J J. Thrombocytopenia. A laboratory sign of septicemia in infants and children. *J Pediatr* 85: 219, 1974.
11. Cowan S T & Steel K J. *Manual for the identification of medical bacteria*. New York, 1974. 2nd ed.
12. Daschner F, Adam D & Marget W. Sepsis und Kontamination bei Nabel und Venenkathetern. *Monatsh Kinderheilkd* 122: 49, 1974.
13. Davies P A. Bacterial infection in the fetus and newborn. *Arch Dis Child* 46: 1, 1971.
14. Dorand E H, Cook L N & Andrews H F. Umbilical vessel catheterization. The low incidence of

- complications in a series of 200 newborn infants
Clin Pediatr 16 569 1977
- 15 Fairchild J P, Graber C D, Vogel F H & Ingersoll R L. Flora of the umbilical stump *J Pediatr* 53 538 1958
 - 16 Gluck L, Wood H F & Fousek M D. Septicemia of the newborn *Pediatr Clin North Am* 13 1131 1966
 - 17 Gotheferns L. Studies of antimicrobial factors in human milk and bacterial colonization of the newborn. Umeå University Med diss 1975
 - 18 Gotoff M P & Behrman R E. Neonatal septicemia *J Pediatr* 76 147 1970
 - 19 Harbel A H, Sandor G S, Conn N K & McCrae W M. Premature rupture of membranes and effects of prophylactic antibiotics *Arch Dis Child* 47 401 1972
 - 20 Kaplan J M, McCracken G H. Bacterial and viral infections of the newborns. In G B Avery (ed) *Neonatology: Pathophysiology and Management of the Newborn*. J P Lippincott Company, Philadelphia 1975 p 542
 - 21 Knudsen F U & Steinrud J. Septicaemia of the newborn associated with ruptured foetal membranes, discoloured amniotic fluid or maternal fever *Acta Paediatr Scand* 65 725 1976
 - 22 Krauss A N, Albert R F & Kannan M M. Contamination of umbilical catheters in the newborn infant *J Pediatr* 77 965 1970
 - 23 Leading article. The special care of human milk. *Br Med J* 11 781 1978
 - 24 Pildes R S, Ramamurthy R S & Vidysagar D. Effect of triple dye on staphylococcal colonization in the newborn infant *J Pediatr* 82 987 1973
 - 25 Powers W F & Tooley W H. Contamination of umbilical vessel catheters. Encouraging information *Pediatrics* 49 470 1972
 - 26 Striuss A W, Escobedo M & Goldring D. Continuous monitoring of arterial oxygen tension in the newborn infant *J Pediatr* 85 254 1974
 - 27 Symansky M E & Fox H A. Umbilical vessel catheterization. Indications, management and evaluation of the technique *J Pediatr* 80 870 1971
 - 28 Tollner U & Pohlndt F. Septicemia in the newborn due to gram negative bacilli. Risk factors, clinical symptoms and hematologic changes *Europ J Pediatr* 123 243 1976
 - 29 van Vliet P K J & Gupta J M. Prophylactic antibiotics in umbilical artery catheterization in the newborn *Arch Dis Child* 48 296 1973
 - 30 Weinstein I. Superinfection. A complication of antimicrobial therapy and prophylaxis *Am J Surg* 107 704 1964
 - 31 Wesstrom G, Finnstrom O & Stenport G. Umbilical artery catheterization in newborns. I. Thrombosis in relation to catheter type and position *Acta Paediatr Scand* 68 375 1979
 - 32 Wilson H D & Eichenwald H F. Sepsis neonatorum *Pediatr Clin North Am* 21 571 1974
 - 33 Yantou M. Leucocyte blood picture in ill newborn babies *Arch Dis Child* 47 741 1972

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UMBILICAL ARTERY CATHETERIZATION IN NEWBORNS

III Thrombosis—A Study of Some Predisposing Factors

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ABSTRACT Henriksson P, Westrom G and Hedner U (Departments of Paediatrics University of Lund Allmänna Sjukhuset Malmö and University Hospital Linköping and The Coagulation Laboratory Allmänna Sjukhuset Malmö Sweden) Umbilical artery catheterization in newborns. III A study of some predisposing factors. *Acta Paediatr Scand* 1979; 68: 719-723. —Thromboses following umbilical artery catheterization is a relatively frequent complication. Low fibrinolytic activity in the vessel walls (high factor VIII and low antithrombin III (AT III) (Heparin cofactor activity) in blood are factors known to favour the formation of thrombosis. In 30 newborns who died and in 2 foetuses the fibrinolytic activity determined in the aorta and the femoral vessels was in the normal adult range except for a few very immature infants and the foetuses. The five cases with arterial thrombosis were not associated with low fibrinolytic activity. The various factor VIII activities (VIII C, VIII R Ag and VIII R RCF) and AT III were studied in 30 sick newborns and in 20 healthy newborns. The sick exhibited increased levels of various factor VIII activities (VIII R Ag and VIII R RCF mainly) and markedly reduced levels of AT III. The high factor VIII activities and the low AT III found will add to the existing risk of thrombosis due to the presence of a foreign material. AT III substitution is suggested as a possible prophylactic.

KEY WORDS Umbilical artery catheterization newborn thrombosis

Thromboembolic complications in connection with umbilical catheterization have received much space in the literature (6, 11, 22, 25, 36). Even spontaneous arterial and venous thrombosis occur in the newborn period, the incidence varying between 1 and 5% (5, 12, 21, 25, 28).

It is generally accepted that low fibrinolytic activity in the vessel walls (19) and a low anti-thrombin III level (7, 20) imply an increased risk of thrombosis. Also an increase in the factor VIII protein is now thought to favour the development of thromboembolic disease (2, 27, 34).

Little is known about the variation of the above mentioned coagulant and fibrinolytic factors and their possible role in thrombosis in the newborn. We therefore thought it worth while to determine the fibrinolytic activity in various vessels in foetuses and infants dying in

the neonatal period and the factor VIII (VIII C, VIII R Ag, VIII R RCF) and anti-thrombin III (AT III) in healthy and sick newborns.

CLINICAL MATERIAL

1 Sick newborns ($n=30$) Four infants had IRDS (hyaline membrane disease), 9 unspecified respiratory symptoms, 10 apnoea due to immaturity and 7 asphyxia. Six infants developed thrombosis following catheterization of an umbilical artery, a condition verified at angiography in 5 and autopsy in 1. Their gestational age ranged between 28 and 43 weeks (mean 34.3). The lowest birth weight was 1000 and the highest 4650 g (mean 2087). A polyvinylchloride umbilical artery catheter was used in all 30 cases.

II Healthy newborns ($n=20$) gestational ages 37-42 weeks (mean 39.6). Range of birth weights 2030-4760 g (mean 3370).

III Neonatal autopsies ($n=30$) Nine infants had IRDS (hyaline membrane disease), 6 apnoea due to immaturity, 9 other pulmonary diseases, 4 congenital malformations and 7 intra-uterine infections. Eighteen infants had an indwelling umbilical artery catheter. Five of them in

cluding one of the sick newborns had arterial thrombosis. The gestational ages ranged from 24 to 42 weeks (mean 32.2). The lowest birth weight was 650 g and the highest 2860 g (mean 2130).

IV This group consisted of two *foetuses* obtained at legal abortion in the 17th and 20th week respectively. Crown-heel lengths 19 and 23 cm.

None of the newborns showed any bleeding symptoms.

METHODS AND SAMPLING

Factor VIII clotting activity (VIII C) according to Nilsson et al (26), *factor VIII related antigen (VIII R Ag)* according to Holmberg & Nilsson (17), *the ristocetin cofactor activity (VIII RCF)* as reported by Zuzel et al (38) and *antithrombin III (ATIII)* with an immunochemical method described by Hedner & Nilsson (13).

Fibrinolytic activity in the vessel walls was determined in the way described by Pandolfi et al (30). The activity is expressed in arbitrary units. Normal range in adult hand veins 6–10.

In the sick newborns blood was obtained on day 1–4 via an indwelling umbilical artery catheter. In the healthy newborns blood was taken from the external jugular vein on day 2–4 with the aid of a vacuumainer system. Informed consent had been given by the parents. Sodium citrate 3.8% dilution 1/9 was used as anticoagulant and plasma was treated as previously described (26). Vessel specimens were obtained from the autopsied infants and the foetuses. The specimens obtained post mortem were taken from the abdominal (L_3-4) and the thoracic part (TH_4-5) of aorta and from the femoral artery and vein. They were frozen in fluid nitrogen and stored in -70°C until examined.

RESULTS

The *fibrinolytic activity* in the aorta from the individual patients is shown in Fig. 1. The foetuses and 8 of the 30 infants who died had low fibrinolytic activity in the aorta. Six of them were born in the 24th to 32nd week. Five infants were low in all vessels and one of them born in the 27th week had arterial thrombosis. No difference in fibrinolytic activity was found

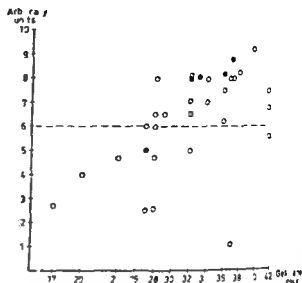


Fig. 1 Fibrinolytic activity in the aorta (arbitrary units) in relation to the gestational age. Filled circles denote cases with arterial thrombosis. Dashed line denotes lower limit in adults.

between the various vessels (Table 1) and no difference could be demonstrated between the proximal and the distal part of the aorta. As is apparent from the figure the fibrinolytic activity did not vary with the presence or absence of thrombosis.

The various activities of factor VIII are shown in Table 2. VIII C, VIII R Ag and VIII RCF in healthy infants significantly exceeded the adult levels ($p < 0.001$, 0.001 and 0.01 respectively). VIII C did not differ significantly between the sick and healthy newborns. VIII R Ag and VIII RCF were however significantly higher in the sick ($p < 0.05$). The various VIII activities did not seem to be influenced by the gestational age.

ATIII also shown in Table 2 was substantially lower in the sick newborns than in

Table 1 Fibrinolytic activity in the vessel wall (arbitrary units: mean and standard deviation)

	n	Thor. aorta	Abdom. aorta	Femoral artery	Femoral vein
Neonatal autopsies	30	6.0 ± 2.3	6.72 ± 2.15	7.05 ± 2.32	6.73 ± 2.68
Foetuses	2	2.75 4.0	— —	— —	5.0 —

Table 2 The various activities of factor VIII (VIII C, VIII R Ag, VIII RCF) and antithrombin III (AT III) (mean and standard deviation)

	VIII C (%)	VIII R Ag (%)	VIII RCF (%)	AT III (%)
Healthy newborns	158.2 ±37.9	138.8 ±79.0	172.6 ±40.0	47.6 ±9.2
Sick newborns	169.9 ±87.3	174.1 ±56.5	147.5 ±37.7	34.3 ±17.6
Healthy adults	96.8 ±76.7	94.0 ±36.1	96.4 ±34.7	103.0 ±10.7

the healthy ($p < 0.001$) who had only half the adult concentration. Infants born before 34th week of gestation had significantly lower AT III levels than those born after that week ($p < 0.05$).

The factor VIII activities and the AT III did not vary with the presence or absence of thrombosis.

DISCUSSION

The development of thrombosis depends on several circumstances. The presence of foreign material implies a risk for platelet adhesion to the foreign surface itself or to collagen exposed by injury of the vessel wall. Furthermore a low fibrinolytic activity in the vessel wall and/or decreased ability to release such activity into the circulation in response to certain stimuli predispose to the development of thrombosis (19). Also conditions in the circulating blood including low inhibitors of coagulation and high levels of certain clotting factors may contribute to any predisposition to thrombotic disease. Thus a decrease of AT III, the inhibitor of thrombin, is known to occur in families with severe thrombotic disease (1, 7, 20, 24). The role of the factor VIII protein in primary haemostasis has recently been clarified. It has been shown that factor VIII is important for the adhesion of platelets to the vessel wall (3, 18, 33) as well as for the formation of large platelet aggregates (39). It has also recently been found that of 148 patients all with recurrent deep venous throm-

bosis factor VIII protein (VIII R Ag) was constantly increased in 29 (20%) (27).

The novel finding of a normal fibrinolytic activity in vessel walls in newborns was in accord with the earlier observation of a normal fibrinolytic activity in blood (8, 10). The development of thrombotic complications did not seem to be related to the fibrinolytic activity in the vessel wall in these infants (Fig. 1). The foetuses and the 6 very immature newborns had however a low fibrinolytic activity indicating a successive development, an observation in agreement with what has been demonstrated for other components of the coagulation and fibrinolytic systems during foetal life (9, 10, 15, 16).

Weststrom *et al.* (36) have recently shown that an umbilical artery catheter carries a significantly greater risk of causing thrombosis when its tip is placed low in the aorta (L_{3-4}) than when it is high ($L_{H_{11}}$). Pandolfi *et al.* (29) have reported a regional variation of the fibrinolytic activity in vessel walls, namely a considerably higher activity in arm veins than in the leg veins, a difference they used to explain why thrombosis occurs predominantly in the legs. As we found no difference between various levels of the aorta, the appearance of thrombosis mainly in the lower part of aorta could not be ascribed to any differences in fibrinolytic activity between different segments.

As for AT III, as expected (32), a decrease by about 50% was found immunochemically in healthy newborns compared with adults. In

the sick newborns the ATIII was decreased even more down to around 30% of the normal adult level. This is in agreement with Weissbach et al (35) who used a biological method for determining ATIII and with Maheshwari & Hathwar (23). The low ATIII could be due to consumption during abnormal proteolysis and/or defective synthesis. Abnormal proteolysis including both inactivation of the coagulation and the fibrinolytic systems has been demonstrated in sick newborns with the same basic diseases as those described in this paper (14).

The low ATIII and the high factor VIII activities even more pronounced in sick newborns might help to explain the thrombotic complications often seen in connection with catheterization of the umbilical arteries. On the other hand the finding of a normal fibrinolytic activity of the vessel walls offers protection against thrombosis and might partly explain the good prospects for those infants who develop thrombosis (4, 31, 37).

Heparin has been suggested as a prophylactic against thrombotic complications also in this age group. The effect of systemic heparin in patients with low ATIII (heparin cofactor activity) is limited. Furthermore it has no inhibitory effect on the first step of the formation of the haemostatic plug and as known it increases the risk of bleeding complications. Whether administration of purified ATIII which is now commercially available (Kabi Stockholm, Sweden) might prove to be the method of choice is a question that must abide future research.

REFERENCES

- 1 Bjarke B, Henn P & Blomback M. Neonatal aortic thrombosis—a possible clinical manifestation of congenital antithrombin III deficiency. *Acta Paediatr Scand* 63: 297, 1974.
- 2 Bloom A L, Giddings J C & Wilks C J. Factor VIII on the vascular intima. Possible importance in haemostasis. *Nature (New Biol)* 241: 217, 1973.
- 3 Bloom A L & Peake I R. Molecular genetics of factor VIII and its disorders. *Semin Haematol* 14: 319, 1977.
- 4 Boros S J, Nystrom J F, Thompson T M, Reynolds J W & Williams H J. Lee growth following umbilical artery catheter-associated thrombus formation. A 4 year follow up. *J Paediatr* 87: 973, 1975.
- 5 Braly B D. Neonatal arterial thrombosis and embolism. *Surgery* 59: 869, 1965.
- 6 Cochran W D, Davis H T & Smith C. Advantages and complications of umbilical artery catheterization in the newborn. *Pediatrics* 47: 69, 1968.
- 7 Egeberg O. Inherited antithrombin deficiency causing thrombophilia. *Thromb Diath Haemorrh* 11: 516, 1965.
- 8 Ekelund H, Hedner U & Nilsson I M. Fibrinolysis in newborns. *Acta Paediatr Scand* 59: 33, 1970.
- 9 Ekelund H, Hedner U & Åstedt H. Fibrinolysis in human fetuses. *Acta Paediatr Scand* 59: 369, 1970.
- 10 Ekelund H & Finnstrom O. Fibrinolysis in preterm infants and in infants small for gestational age. *Acta Paediatr Scand* 61: 185, 1972.
- 11 Goetzman B W, Stadnick R C, Bogren H G, Blankenship W J, Ikeda R M & Thayer J. Thrombotic complications of umbilical artery catheters. A clinical and radiographic study. *Pediatrics* 56: 374, 1975.
- 12 Gross R E. Arterial embolism and thrombosis in infancy. *Am J Dis Child* 70: 61, 1945.
- 13 Hedner U & Nilsson I M. Antithrombin III in a clinical material. *Thrombosis Research* 3: 631, 1973.
- 14 Henriksson P & Ekelund H. Abnormal proteolysis in sick newborns. *Acta Paediatr Scand* 64: 377, 1975.
- 15 Henriksson P, Hedner U, Nilsson I M, Boehm J, Robertson M & Lorand L. Fibrin stabilizing factor (Factor XIII) in the fetus and the newborn infant. *Leidat Res* 8: 789, 1974.
- 16 Holmberg L, Henriksson P, Ekelund H & Åstedt H. Coagulation in the human fetus. *J Pediatr* 83: 860, 1974.
- 17 Holmberg L & Nilsson I M. Immunologic studies in haemophilia A. *Scand J Haematol* 10: 17, 1973.
- 18 Hovig T & Stormorken H. Ultrastructural studies on the platelet plug formation in bleeding time wounds from normal individuals and patients with von Willebrand's disease. *Acta Pathol Microbiol Scand Suppl* 249: 105, 1974.
- 19 Isacson S & Nilsson I M. Defective fibrinolysis in blood and vein walls in recurrent idiopathic venous thrombosis. *Acta Chir Scand* 138: 313, 1977.
- 20 Johansson L, Hedner U & Nilsson I M. Familial antithrombin III deficiency as pathogenesis of deep venous thrombosis. *Acta Med Scand* 204: 491, 1978.
- 21 Knowlson G T & Marsden H B. Aortic thrombosis in the newborn period. *Arch Dis Child* 53: 164, 1978.
- 22 Larroche J Cl. Umbilical catheterization: its complications. *Biol Neonate* 16: 101, 1970.
- 23 Mahasandana C & Hathaway W. Circulating anticoagulants in the newborn: relation to hypercoagulability and the idiopathic respiratory distress syndrome. *Pediatr Res* 7: 670, 1973.
- 24 Marciniak E, Farley C H & de Simone P A.

- Familial thrombosis due to antithrombin III deficiency *Blood* 43 719 1974
- 6 Neal W A Reynolds J W Jarvis C W & Williams M J Umbilical artery catheterization Demonstration of arterial thrombosis by angiography *Pediatrics* 50 11 1972
- 6 Nilsson I M Blomback M & von Francken I On an inherited autosomal haemorrhagic diathesis with antithrombophilic globulin (AHG) deficiency and prolonged bleeding time *Acta Med Scand* 159 35 1957
- 7 Nilsson I M Hedner U & Nilsson B Factor VIII in thrombosis and intravascular coagulation Vth Congress on Thromboembolism Bologna 1978
- 78 Oppenheimer E H & Esterly J R Thrombosis in the newborn Comparison between infants of diabetic and nondiabetic mothers *J Pediatr* 67 549 1965
- 9 Pandolfi M Nilsson I M Robertson B & Isacson S Fibrinolytic activity of human veins *Lancet* ii 177 1967
- 30 Pandolfi M Isacson S & Nilsson I M Low fibrinolytic activity in the walls of veins in patients with thrombosis *Acta Med Scand* 186 1 1969
- 31 Powers W F & Swyer P R Limb blood flow following umbilical arterial catheterization *Pediatrics* 55 748 1975
- 3 Teger Nilsson A C Antithrombin in infancy and childhood *Acta Paediatr Scand* 64 674 1975
- 33 Tschopp T B Weiss H J & Baumgartner H R Decreased adhesion of platelets to subendothelium in von Willebrand's disease *J Lab Clin Med* 83 796 1974
- 34 Weiss H J Platelet physiology and abnormalities of platelet function *N Engl J Med* 293 580 1975
- 35 Weissbach G Domula M Lenk H & Schneider P The progressive antithrombin activity and its relations to other factors of the coagulation system in newborns *Acta Paediatr Scand* 67 555 1974
- 36 Weststrom G Finnstrom O & Stenport G Umbilical artery catheterization in newborns I Thrombosis in relation to catheter type and position *Acta Paediatr Scand* 68 575 1979
- 37 Weststrom G Umbilical artery catheterization in newborns V A follow up study To be published
- 38 Zuzel M Nilsson I M & Åberg M A method for measuring plasma ristocetin cofactor activity Normal distribution and stability during storage *Thrombosis Research* 12 745 1978
- 39 Åberg M & Rausing A The effect of Dextran 70 on the structure of ex vivo thrombi *Thrombosis Research* 12 1113 1978

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GASTRIC EMPTYING IN PRETERM INFANTS

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ABSTRACT Cavell B (Department of Paediatrics University of Lund Lund Sweden) Gastric emptying in preterm infants. *Acta Paediatr Scand* 68 725 1979.—The gastric emptying of meals of human milk and infant formula was studied in 11 healthy preterm (AGA) infants at a postnatal age of 1–9 weeks corresponding to 33–38 weeks of gestational age. A total of 30 studies were performed using a marker dilution technique. Gastric emptying of meals of human milk followed a biphasic emptying pattern with an initial fast phase. In about 1/2 min half of the meal had left the stomach. The emptying of meals of adapted infant formula usually followed a linear pattern and half the meal had left the stomach after 51 min. After one hour an average of 24.8 ml of human milk and 22 ml of infant formula had left the stomach.

KEY WORDS Gastric emptying, preterm infants

In recent years there has been a growing interest in the feeding of the preterm infant. Various techniques in providing these infants with an adequate nutritional supply e.g. naso-gastric and transpyloric feeding have been suggested. There are however reports on intestinal complications in preterm infants receiving transpyloric feedings (4, 8, 14). Nasogastric feeding is not associated with such complications. This technique carries however the risk of regurgitation and aspiration of gastric contents when the volume of the meal exceeds the capacity of the infant's stomach for handling it. On the other hand too small meal volumes may not fulfill the nutritional demands of the infant. Knowledge of the reservoir function of the stomach of the preterm infant would thus seem important. The purpose of the present study was to estimate quantitatively the gastric emptying function in healthy preterm infants fed complete meals

from the first day of the mother's last menstrual period. The degree of maturity was furthermore estimated by assessing external features: neurological development and head circumference (5). Body surface was estimated according to Haycock et al. (7). All infants were appropriate in weight for gestational age (AGA) according to Swedish standards (18). At the time of the studies all the infants were healthy and growing normally. They were nursed in incubators and fed through a nasogastric infant feeding tube. The studies were made after informed consent of the mothers.

METHODS

General procedure. Each infant was studied on one to five occasions making a total of 30 studies in the eleven infants. The infants were given lukewarm test meals of fresh frozen human milk usually obtained from their own mother or an adapted infant formula. The composition of the infant formula used is shown in Table 2. The volume of the test meal was about 27 ml/kg body weight corresponding to the volume of the infant's ordinary meal.

Part of this work was presented at The Joint Meeting of the European Society for Pediatric Gastroenterology and Nutrition & The North American Society for Pediatric Gastroenterology in Paris, May 4–6, 1978 (3).

Abbreviations. IRDS = idiopathic respiratory distress syndrome. RIS = respiratory insufficiency syndrome (15). TT = transient tachypnoea. IPPV = intermittent positive pressure ventilation. CPAP = continuous positive airway pressure. ET = exchange transfusion.

MATERIAL

Eleven preterm infants were studied at a postnatal age of one to nine weeks. The clinical data on the infants are presented in Table 1. Gestational age was calculated

Table 1 *Clinical data in preterm infants*

For abbreviations see text

Patient no	Sex	Birth weight (g)	Gestational age at birth (weeks)	Previous history
1	f	950	29	Chyllothorax IPPV 7 days CPAP 2 days
2	f	1 800	32	Twin TT
3	f	1 815	32	Twin
4	m	1 940	34	
5	f	960	29	RIS IPPV 9 days CPAP 2 weeks
6	f	910	28	IRDS IPPV 19 days CPAP 4 days
7	f	1 020	31	
8	m	900	28	IRDS RIS IPPV 6 weeks CPAP 3 hours
9	f	1 555	32	TT
10	f	1 280	32	Twin IRDS IPPV 1 day CPAP 4 days Hyperbilirubinaemia ET
11	f	1 350	32	Twin IRDS CPAP 5 days

A French size 5 infant feeding tube was introduced into the stomach through the nose. The gastric contents were withdrawn and the stomach was washed out with saline immediately before the test meal was injected. During the study the infant was lying prone or in the right lateral position in an incubator.

Amount of test meal in the stomach. The amounts of test meal remaining in the stomach after various intervals were estimated using a modification of an earlier described procedure (2). A marker dilution technique is used after the infant has received a test meal containing polyethylene glycol (PEG Microgol 3000). To an aliquot of the test meal is added a specified amount of PEG. This aliquot is introduced into the stomach and mixed with the gastric contents by withdrawing and reinserting with a syringe for 2 min. Immediately before and after this procedure samples are withdrawn from the stomach for the estimation of PEG concentration.

The first volume determination was thus performed 10–20 min after feeding had started. The procedure was

repeated once or twice at various intervals during the study.

The volume of the gastric contents V is calculated from the equation

$$V = V_2 \frac{(C - C_2)}{(C_2 - C_1)} + V_1$$

where C = PEG concentration of test meal aliquot V_2 = volume of test meal aliquot C_1 = PEG concentration of gastric contents before adding test meal aliquot C_2 = PEG concentration of gastric contents after adding test meal aliquot and V_1 = volume of sample of gastric contents withdrawn immediately before adding test meal aliquot. The amount of test meal remaining in the stomach is estimated using the volume calculated according to the equation above and the changes of the PEG concentration during the study.

After 50 to 100 min the procedure was finished by withdrawing the remaining gastric contents and washing out the stomach with 5 ml of saline. The total residue of the meal was calculated using the PEG concentrations of the recovered gastric contents and of the wash out.

The gastric quarter half and three-quarter-emptying times i.e. the time required to empty 25, 50 and 75% of the test meal respectively were obtained by interpolation from individual emptying curves.

Analytical method. PEG was estimated turbidimetrically by the method of Hyden (10) using the modifications by Boulter & McMichael (1).

Test of validity. The validity of the marker dilution technique in estimating known amounts of test meals of infant formula was tested *in vitro*. A small amount of 0.1 molar hydrochloric acid was added to a vessel containing a known amount of infant formula and PEG. After mixing the amount of infant formula in the vessel was estimated using the procedure described above. A part of the contents of the vessel was removed, another small amount of hydrochloric acid was added and the amount of infant formula remaining in the vessel was again estimated. The

Table 2 *Data on infant formula*

Energy content	2 900 kJ/l
Osmolality	290 mOsm/kg
<i>Composition</i>	
Protein	16
Casein	6.5
Whey proteins	9.5
Fat	35
Lactose	72
Sodium	0.2
Potassium	0.7
Calcium	0.4
Phosphorus	0.76

Fat is derived from lard, palm oil, cottonseed oil and sunflower oil. The amount of linoleic acid corresponds to 6% of the total energy content.

Table 3 Weighed versus experimentally estimated amounts of test meal *in vitro*

Estimation no	Test meal (ml)		Difference (ml)
	Weighed	Estimated	
1	79.1	8.6	0.5
2	19.7	17.1	2.6
3	11.4	11.9	-0.5
4	7.6	7.1	0.5
5	4.3	3.9	0.4

procedure was repeated three more times. The amounts added to or removed from the vessel as well as the vessel including the test meal were weighed accurately. The results of this test are presented in Table 3.

Statistical methods: Student's *t* test, linear regression analysis and the Fisher exact probability test were used in the statistical analysis.

RESULTS

Emptying patterns: In 27 of the 30 studies gastric emptying had started at the first volume determination. A biphasic emptying pattern with an initial rapid phase lasting some 20 min followed by a slower phase was found in 19 of these 27 studies. The emptying rate appeared constant from the start in 5 studies including 4 performed with infant formula. In studies (2 with human milk) only small amounts had emptied at the first determination. The subsequent emptying was faster.

In two of the three remaining studies both with infant formula no emptying had occurred at the first determination 16 and 21 min after feeding respectively. Following this initial delay gastric emptying proceeded with a constant rate in one study while in the other study the emptying curve was biphasic with an initial rapid phase.

In the remaining study it was not possible to draw conclusions about the initial emptying as the first volume determination was invalidated by improper positioning of the feeding tube.

In 16 studies where meals of human milk and infant formula were given to the same infant the biphasic emptying pattern with an initial fast phase was observed significantly

more often than any of the other emptying patterns after meals of human milk than after meals of infant formula ($p = 0.024$).

The biphasic emptying pattern is illustrated in Fig. 1 which shows the gastric emptying in 4 studies performed in one infant.

Gastric emptying times: The gastric emptying times for one quarter, half and three quarters respectively of a test meal are presented individually in Table 4. The means and standard deviations of these emptying times are shown in Table 5.

The quarter, half and three quarter-emptying times for meals of infant formula were significantly longer than for meals of human milk ($p < 0.001$).

No relationship was found between the time needed to administer the test meal and the gastric quarter and half emptying times.

Gastric emptying rate: Sixty minutes after administration of the meal 24.8 ± 7.5 ml of human milk meals and 19.2 ± 4.7 ml of infant formula meals had left the stomach. The difference is significant ($p < 0.05$).

The corresponding figures calculated per 0.1 m^2 of body surface area are 19.4 ± 4.5 ml and 13.8 ± 2.8 ml respectively. The difference is significant ($p < 0.01$).

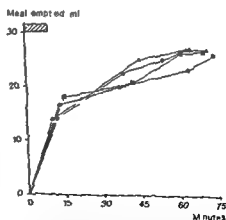


Fig. 1 Gastric emptying in a 5 week-old preterm infant (patient no. 6) after 30 ml test meals of human milk. The amount of meal that has left the stomach is plotted against time. Results are from four different studies performed on four consecutive days. The shaded area indicates the mean duration of the administration of the meals.

Table 4 Gastric emptying times in 11 healthy preterm infants

H = human milk F = infant formula lat = right lateral

Patient no	Age at time of study (weeks)	Body weight at time of study (g)	Body position	Test meal (ml)	Time for injecting test meal (min)	Time in min for emptying of specified fraction of test meal		
						25%	50%	75%
1	5	1 380	Lat	30 H	12	8	16	41
			Lat	30 H	12	8	15	23
			Lat	30 H	12	8	31	51
2	2	1 760	Lat	40 F	4	22	40	95
3	2	1 820	Lat	40 F	6	28	49	74
4	2	1 900	Lat	60 H	6	16	32	66
			Lat	50 H	4	12	23	33
5	11	1 600	Lat	30 H	8	7	30	40
		1 780	Lat	35 H	10	5	11	16
			Lat	35 H	15	24	36	79
6	4	1 180	Lat	30 H	9	7	15	36
	5	1 270	Prone	30 H	9	5	16	19
			Prone	30 H	12	7	13	49
			Prone	30 H	10	6	12	49
			Prone	30 F	11	36	44	79
			Prone	30 F	11	36	44	79
7	2	1 073	Lat	25 H	7	11	36	44
	3	1 180	Lat	25 H	8		44	61
			Lat	30 H	10	6	11	21
8	6	1 390	Prone	25 H	7	14	27	40
	7	1 400	Prone	25 H	15	20	30	60
			Prone	25 F	5	28	48	86
9	1	1 450	Lat	25 H	7	18	34	64
10	2	1 410	Prone	25 H	12	8	16	64
	3	1 510	Prone	30 H	16	35	52	78
			Lat	30 F	13	34	48	76
	4	1 760	Prone	40 F	11	27	60	97
11	2	1 420	Prone	25 H	8	8	17	48
	3	1 570	Lat	30 H	15	12	32	71
			Prone	30 F	11	53	64	79
	4	1 760	Prone	40 F	9	12	32	74

Gastric emptying rate and postconceptional age of the infant were not significantly correlated ($r=0.31$ $p>0.05$). The rate of emptying was however related to the infant's body surface area ($r=0.43$ $p<0.02$) and body weight ($r=0.40$ $p<0.05$).

DISCUSSION

Comparison of methods Accurate data on gastric emptying of complete meals in the newborn and young infant are scarce. Earlier investigators have used radiological techniques in assessing gastric emptying of milk meals

containing barium in infants (16). Because radiation hazards and poor correlation with more accurate methods (6) these techniques are however unsuitable.

Gastric emptying has been studied in infants using the serial test meal (9). This technique does not however allow one to follow the whole emptying course of a single meal. It is possible using radioisotope techniques (17).

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Table 5 Gastric emptying of human milk and infant formula in preterm infants

Mean values \pm S.D.

Test meal	Gastric emptying time (min) Percent of test meal emptied		
	25	50	75
Human milk	11.7 \pm 7.5 (n=7)	25.1 \pm 11.5 (n=7)	51.3 \pm 16.7 (n=7)
Infant formula	30.0 \pm 11.9 (n=8)	51.9 \pm 9.8 (n=8)	80.0 \pm 10.7 (n=8)

marker dilution technique is demonstrated by the *in vitro* test where calculated amounts agree closely with known amounts.

PEG has been used extensively as a volume indicator in studies of intestinal absorption and has also proved to be valid as a gastric non-absorbable indicator for studies in man (11).

Gastric emptying pattern The biphasic emptying pattern which was found in most of the studies occurred significantly more frequently after meals of human milk than after meals of infant formula which usually were associated with a linear emptying pattern or with an initial delay of gastric emptying. The differences in emptying of the meals appear to be due to differences in the composition of human milk and infant formula.

Gastric emptying times Gastric emptying times were short (Table 5). The differences in the emptying patterns between the two meals is reflected by the different gastric emptying times which were significantly longer for meals of infant formula than for meals of human milk.

The half time of the meal in the stomach observed in the present study is considerably shorter than the figure reported by Signer & Fridrich (17) in infants given a standard meal of infant formula. This may at least in part be due to different meal volumes and different body positions being used in the two studies (19).

Gastric emptying rate The average amount of meal that had left the stomach during the

first 60 min of a study was significantly larger for meals of human milk than for meals of infant formula. Keller (12) in a study on gastric emptying of human milk in infants found that the gastric emptying rate was constant and proportional to the infant's body surface area corresponding to an emptying of 22 ml per 0.1 m² body surface area per hour. The emptying rate for meals of human milk found in the present study agrees fairly well with this figure.

Conclusion The present study shows that healthy preterm infants even if weighing slightly but above 1000 g have a rapid gastric emptying after a feed of human milk. Gastric emptying times for meals of infant formula were roughly twice as long as those for meals of human milk.

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REFERENCES

- Boulter J M & McMichael H B. Modification of poly-ethylene glycol estimation suitable for use with small animals. *Gut* 11: 268, 1970.
- Cavelti B. Gastric emptying in infants. *Lancet* 11: 904, 1969.
- Cavelti B. Gastric emptying in low birth weight infants. *Acta Paediatr Belg* 31: 176, 1978.
- Chen J W & Wong P W K. Intestinal complications of nasogastric feeding in low birth weight infants. *J Pediatr* 85: 109, 1975.
- Finnstrom O. Studies on maturity in newborn infants. VI. Comparison between different methods for maturity estimation. *Acta Paediatr Scand* 61: 33, 1972.
- Fridrich R, Sulder G A, Locher J & Heinis P. Zur Erweiterung der Magendiagnostik mit nuklearmedizinischen Verfahren. *Dtsch Med Wochenschr* 95: 761, 1970.
- Haycock G B, Schwartz G J & Wisotsky D H. Geometric method for measuring body surface area: A height weight formula validated in infants, children and adults. *J Pediatr* 93: 63, 1978.

Table 4 Gastric emptying times in 11 healthy preterm infants

H = human milk F = infant formula Lat = right lateral

Patient no	Age at time of study (weeks)	Body weight at time of study (g)	Body position	Test meal (ml)	Time for injecting test meal (min)	Time in min for emptying of specified fraction of test meal		
						25%	50%	75%
1	5	1380	Lat	30 H	12	8	16	57
			Lat	30 H	12	8	15	73
			Lat	30 H	12	8	37	57
2	2	1780	Lat	40 F	4	22	50	95
3	2	1870	Lat	40 F	6	28	49	74
4	2	1900	Lat	60 H	6	16	37	66
			Lat	50 H	4	12	77	33
5	8	1600	Lat	30 H	8	7	30	50
	9	1780	Lat	35 H	10	5	12	76
			Lat	35 H	15	24	36	79
6	4	1180	Lat	30 H	9	7	15	36
	5	1270	Prone	30 H	9	5	16	39
			Prone	30 H	12	7	13	49
			Prone	30 H	10	6	17	59
			Prone	30 F	11	36	54	79
7	2	1075	Lat	25 H	7	11	36	54
	3	1180	Lat	25 H	8		44	67
			Lat	30 H	10	8	17	27
8	6	1390	Prone	25 H	7	14	27	40
	7	1500	Prone	25 H	15	20	30	60
			Prone	25 F	5	29	48	86
9	1	1450	Lat	25 H	7	18	34	64
10	2	1410	Prone	25 H	12	8	16	64
	3	1510	Prone	30 H	16	35	52	78
			Lat	30 F	13	34	58	76
	4	1760	Prone	40 F	11	27	60	97
11	2	1420	Prone	25 H	8	8	17	48
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SMOKING DURING PREGNANCY—HEMATOLOGICAL OBSERVATIONS
IN THE NEWBORN

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ABSTRACT Meberg A Hågå P Sande H and Foss O P (Departments of Paediatrics Obstetrics and Gynaecology Clinical Chemistry and Pathology Ullevål Hospital Oslo Norway) Smoking during pregnancy—hematological observations in the newborn Acta Paediatr Scand 68 731 1979—Hematological values were measured in 28 newborn infants of mothers smoking 10–20 cigarettes daily during pregnancy and in 25 infants of non-smokers Higher hematocrit levels were found on the 1st day of life in infants of smoking mothers (60.8 ± 5.0 mean \pm SD) compared to controls (57.5 ± 4.8) ($p < 0.05$) The hematocrit levels correlated positively with the maternal smoking level ($r = 0.318$ $p < 0.04$) and the maternal serum thiocyanate concentrations at delivery ($r = 0.389$ $p < 0.01$) Cord serum values for erythropoietin serum iron transferrin and ferritin showed no statistically significant difference between the two groups A significant inverse correlation was found between the hematocrit value on the 1st day of life and the cord serum ferritin concentration ($r = -0.495$ $p < 0.005$) The present results suggest that maternal smoking stimulates fetal erythropoiesis probably through a hypoxic effect on the fetus dose related to the maternal smoking level Increased erythropoiesis may cause increased iron incorporation into erythrocytes at expense of iron storage in the bone marrow and reticuloendothelial system

KEY WORDS Maternal smoking newborn infants hematocrit thiocyanate erythropoiesis ferritin

Maternal cigarette smoking during pregnancy may create a condition of chronic hypoxia for the fetus This may be due to impaired oxygen transport capacity because of carboxyhemoglobin production in mother and fetus (3) Carbon monoxide also causes a shift to the left of the oxyhemoglobin dissociation curve (3) impairing release of oxygen to the tissues Ultrastructural changes in the placenta (2) as well as decreased placental blood flow (17) may also impair oxygen supply to the fetus in smoking individuals Increased hemoglobin concentrations and elevated hematocrit levels were recently reported in infants of smoking mothers (4) In association with intrauterine growth retardation (IUGR) we have found transitory postnatal thrombocytopenia more frequently among infants of smoking mothers than of non smokers (13)

The purpose of the present investigation was to investigate hematocrit reticulocyte and thrombocyte counts erythropoietin (ESF) serum iron transferrin and ferritin levels in newborn infants of smoking and non smoking mothers and to relate these observations to the maternal smoking level

MATERIALS AND METHODS

Fifty three mothers (28 smoker and 25 non smokers) and their newborn infants were included in the series Consecutive smoking mothers were selected if they fulfilled the following criteria healthy women aged between 20 and 30 years in their first or second uncomplicated pregnancy a normal delivery at 39–42 weeks of gestation and a cigarette consumption estimated by the women themselves to be 10 15 or 20 cigarettes daily for the whole pregnancy The non smoking mothers selected were the first non smoking women admitted for delivery after a mother in the smoking group and that fulfilled all the other selection criteria Twenty five smokers and 23 non

- 8 Heird W C Nasogastric feeding. A commentary *J Pediatr* 85 111 1974
- 9 Husband J & Husband P Gastric emptying of water and glucose solutions in the newborn *Lancet* II 409 1969
- 10 Hydén B A turbidimetric method for the determination of higher polyethylene glycols in biological materials *Ann Roy Agric Coll Sweden* 22 139 1956
- 11 Ivey K J & Schedl H P Gastric nonabsorbable indicators for studies in man *Gastroenterology* 59 234 1970
- 12 Keller W Studies of the rapidity with which food leaves the stomach of infants *Acta Paediatr Scand* 35 180 1948
- 13 Landwirth J Continuous nasogastric infusion versus total intravenous alimentation *J Pediatr* 81 1037 1972
- 14 Pérez Rodrigues J Quero J Frias E G & Omeñaca F Duodeno renal perforation in a neonate by a tube of silicone rubber during transpyloric feeding *J Pediatr* 92 113 1978
- 15 Rishi N C R Experience with neonatal intensive care in Finland *Ross Conference on Pediatric Research* 59 64 1968
- 16 Schnell N B Karelitz S & Epstein B S Radiographic study of gastric emptying in premature infants *J Pediatr* 67 342 1963
- 17 Signer E & Frödrich R Gastric emptying in newborns and young infants *Acta Paediatr Scand* 64 525 1975
- 18 Sterky H Swedish standard curves for intrauterine growth *Pediatrics* 46 7 1970
- 19 Yu V Y H Effect of body position on gastric emptying in the neonate *Arch Dis Child* 50 500 1975

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Table 1 Hematocrit levels, reticulocyte and thrombocyte counts in capillary blood samples on the 1st and the 4th day of life in infants of smoking and non smoking mothers

Cigarette consumption ranged 10-20 cigarettes daily during pregnancy in the smoking group. n = number of observations

	Day after birth	Smokers			Non smokers			p
		n	Range	Mean \pm S D	n	Range	Mean \pm S D	
Hematocrit (%)	1	25	49-75	60.8 \pm 5.0	23	49-67	57.5 \pm 4.8	<0.05
	4	22	52-68	59.9 \pm 5.2	20	47-66	56.4 \pm 5.6	>0.05
								<0.10
Thrombocytes ($\times 10^9/l$)	1	25	187-405	290 \pm 54	23	162-363	285 \pm 47	>0.05
	4	23	160-395	286 \pm 67	20	152-360	273 \pm 52	>0.05
Reticulocytes (%)	1	25	1.3-3.1	2.2 \pm 0.41	22	1.5-2.9	2.2 \pm 0.34	>0.05
	4	23	0.5-1.9	1.0 \pm 0.3	18	0.5-1.3	0.9 \pm 0.3	>0.05
Decrease in reticulocyte number (%)	1-4	22	0.5-2.0	1.2 \pm 0.4	18	0.6-1.9	1.3 \pm 0.3	>0.05

smokers used iron supplements and 25 smokers and 21 non smokers used a multivitamin preparation during pregnancy according to a routine regimen. After delivery, cords were clamped when pulsations in the umbilical cord arteries stopped.

Blood samples were taken from the neonates by heel punctures on the 1st and 4th day of life. The samples were analyzed for microhematocrit, reticulocyte (1000 red cells counted after incubation with 1% brilliant cresyl blue) and thrombocyte counts (phase contrast microscopy). Cord blood samples were rapidly centrifuged and serum stored at -30°C until analyzed for serum iron, transferrin, ferritin and ESF. Serum iron was measured on a Technicon[®] autoanalyzer with the technique adjusted for 180 μl serum. Transferrin was measured by single radial immunodiffusion technique (M. Partigen[®], Behring Institut, Germany) and ferritin by an immunoradiometric assay (Ria Gnost Ferritin[®], Behring Institut, Germany). ESF in cord serum was determined by a cell culture assay (5) measuring the number of erythroid colonies (CFU) formed in response to serum added to the cultures. The serum concentration used was 100 $\mu\text{l}/\text{ml}$ and all determinations were done in duplicate. A step III preparation of sheep plasma ESF (Connaught Laboratories, Willowdale, Canada) was used as the standard.

Venous blood samples were taken from the mothers less than 24 hours before delivery. The samples were rapidly centrifuged and the serum stored at -30°C until analyzed for thiocyanate concentration by an automated modified method after Pettigrew & Fell (14). Informed consent from the mother was obtained in every case before blood samples were collected from them and their infants.

All blood samples were coded before analysis for every type of determination performed.

RESULTS

Table 1 shows the hematocrit, reticulocyte and thrombocyte counts in the newborn in

infants on the 1st and 4th day of life. Infants of smoking mothers had increased hematocrit levels on the 1st day of life compared to infants of non smokers ($p < 0.05$). The same differences were present on the 4th day of life with borderline significance ($0.10 > p > 0.05$). No differences were found in the reticulocyte and thrombocyte counts and the fall in reticulocyte count from the 1st to the 4th day of life.

Maternal serum thiocyanate concentration at delivery (mean \pm S D) was significantly elevated in smokers compared to non smokers (109.6 \pm 29.9 $\mu\text{mol}/\text{l}$ and 48.2 \pm 16 $\mu\text{mol}/\text{l}$ respectively, $p < 0.01$) and the thiocyanate concentration correlated positively to the maternal smoking level ($r = 0.80$, $p < 0.001$). The infants' 1st day hematocrit values were found to correlate positively with the serum thiocyanate concentrations of the mothers (Fig. 1). A positive correlation was also found between the mothers' estimated cigarette consumption and their infants' 1st day hematocrit values ($r = 0.318$, $p < 0.05$).

No statistically significant differences were found between the infants of smoking and non smoking mothers with regard to cord serum concentrations of serum iron (28.6 \pm 6.9 vs 29.2 \pm 5.5 $\mu\text{mol}/\text{l}$), transferrin (2.21 \pm 0.38 vs 2.22 \pm 0.30 g/l), ferritin (mean 276 vs 274 ng/ml , geometric mean \pm S D 157-484 vs 120-

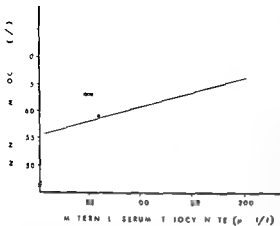


Fig 1 Relation between maternal serum thiocyanate concentrations at delivery and the hematocrit values of their infants on the 1st day of life in 3 smoking and 23 non smoking mothers

622 ng/ml) and ESF (980 ± 351 vs 787 ± 372 CFU/ 2×10^6 cells)

A highly significant inverse correlation was found between the infants 1st day hematocrit values and their cord serum ferritin concentrations (Fig 2)

DISCUSSION

The increased hematocrit values found in infants of smoking mothers on the 1st day of life is in accordance with the findings of Garn & Shaw (4). This fits the hypothesis that maternal smoking creates a hypoxic condition for the fetus stimulating erythropoiesis.

In adults a positive correlation is found between the hematocrit level and cigarette consumption (6). Thiocyanate is a detoxication product of cyanide absorbed from the smoke and is found to reflect the maternal smoking level (1, 12). The hematocrit levels of neonates increased with increasing maternal serum thiocyanate concentration (Fig 1) and also with increasing cigarette consumption. This indicates that the higher the maternal smoking level the stronger the hypoxic stimulus for fetal erythropoiesis.

Hypoxia is a stimulus for ESF production. During chronic hypoxia however serum ESF

levels decrease towards pre hypoxic levels after an initial increase (15). Fetal growth may also be a potent stimulus for erythropoiesis (9). In infants with IUGR we have found that cord serum ESF levels were not different from the ESF levels in infants with birth weights appropriate for gestational age though IUGR infants had elevated hemoglobin concentrations (10). Chronic intrauterine hypoxia and a somewhat decreased growth rate of the fetus in smoking mothers therefore may be in accordance with normal cord serum ESF levels in these infants.

Polycythemia is found in cigarette smokers with increase of the absolute red cell volume (16). In some individuals however a decreased plasma volume is found causing a relative polycythemia. From the present data it cannot be excluded that a decreased plasma volume may contribute to the elevated hematocrit values found in newborn infants of smoking mothers.

It has been suggested that infants of smoking mothers carry a larger iron reserve into the postnatal period than infants of non smokers (4). This may be correct as most of the infants iron reserves are contained in the red blood cells iron stores in the bone marrow and the

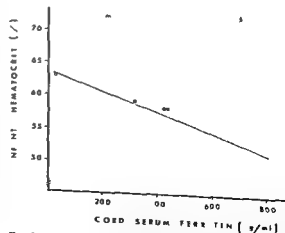


Fig 2 Relation between hematocrit values on the 1st day of life in 35 infants and their cord serum ferritin concentrations. Seventeen of the infants had mothers smoking 10-20 cigarettes daily during pregnancy

reticuloendothelial system are reflected in the serum ferritin levels (8). As far as ferritin is concerned we found no difference in these iron stores in infants of smokers and non smokers. This was also the case with regard to the serum iron and the transferrin concentrations in cord serum.

The inversely found between the infants hematocrit values on the 1st day of life and their cord serum ferritin concentrations (Fig 2) suggests that increased red cell production may cause iron incorporation into erythrocytes at expense of iron storage in the bone marrow and the reticuloendothelial system.

IUGR is often caused by a placental dysfunction syndrome with impaired oxygen supply to the fetus. High hematocrit levels and a transitory postnatal thrombocytopenia frequently occur in these infants (13). The thrombocytopenia seems to be related to chronic intrauterine hypoxia (11) probably because of a competition on common stem cells for erythropoiesis and thrombopoiesis (7). In fetuses suffering from a placental dysfunction syndrome maternal smoking may cause an additional hypoxic stress. This may explain why low thrombocyte counts more frequently occurred in neonates with IUGR of smoking mothers than in IUGR infants of non smokers (13). In normal pregnancy however the hypoxic stress on the fetus from maternal smoking may not exceed the compensatory mechanisms for thrombopoiesis.

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REFERENCES

- Andrews J. Thiocyanate and smoking in pregnancy. *Br J Obstet Gynaecol* 80: 810, 1973.
- Asmussen I. Ultrastructure of the human placenta at term. Observations on placentas from newborn children of smoking and non smoking mothers. *Acta Obstet Gynecol Scand* 56: 119, 1977.
- Cole P V, Hawkins L H & Roberts D. Smoking during pregnancy and its effects on the fetus. *Br J Obstet Gynaecol* 79: 782, 1977.
- Garn S M & Shaw H A. Effect of maternal smoking on hemoglobins and hematocrits of the newborn. *Am J Clin Nutr* 31: 557, 1978.
- Häggl P & Falkanger B. In vitro assay for erythropoietin. Erythroid colony formation in methylcellulose used for the measurement of erythropoietin in plasma. *Blood* in press.
- Isager H & Hagerup L. Relationship between cigarette smoking and high packed cell volume and hemoglobin levels. *Scand J Haematol* 8: 241, 1971.
- Jackson C W & Edwards C C. Biphasic thrombopoietic response to severe hypobaric hypoxia. *Br J Haematol* 35: 233, 1977.
- Lanzkowski P. Iron metabolism in the newborn infant. *Clin Endocrinol Metabol* 5: 149, 1976.
- Maloth Y & Zaizov M. Effect of intrauterine growth on erythropoiesis. *Exp Hematol* 6: 451, 1978.
- Meberg A. Unpublished data.
- Meberg A & Halvorsen S. Transitory thrombocytopenia in small for dates infants and in newborn mice exposed to hypobaric hypoxia, cigarette smoke and CO gas inhalation during pregnancy. *Proc Europ Soc Paediatr Res Annual Meeting* Turku 1978, p. 58.
- Meberg A, Sande H, Foss O P & Stenwig J T. Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby. *Acta Paediatr Scand* in press.
- Meberg A, Ørstavik I & Søyde A. Erythroblastemia and thrombocytopenia in small for gestational age infants. Relation to intrauterine hypoxia, infections and maternal smoking. *Acta Paediatr Belg* 31: 213, 1978.
- Pettigrew A M & Fell G S. Simplified colorimetric determination of thiocyanate in biological fluids and its application to investigation of the toxic amblyopias. *Clin Chem* 18: 996, 1972.
- Schooley J C & Mahlmann L J. Hypoxia and the initiation of erythropoietin production. *Blood Cells* 1: 429, 1975.
- Smith J R & Landaw N A. Smokers polycythemia. *N Engl J Med* 298: 6, 1978.
- Spira A, Fournier R, Grob J C, Dreyfus J & Schwartz D. Smoking during pregnancy and placental blood flow. *Proc 6th Europ Congress of Perinatal Medicine* Vienna 1978.

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INSULIN AND GLUCAGON SECRETION IN HEPATIC GLYCOGENOSES

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ABSTRACT Okada S Seino Y Kodama H Yutaka T Inui K Ishida M Yabuuchi II and Seino Y (Department of Pediatrics Osaka University Hospital Fukushima ku Osaka and The Third Division Department of Medicine University of Kobe Kobe Japan) Insulin and glucagon secretion in hepatic glycogenoses Acta Paediatr Scand 68 735 1979 — Insulin and glucagon secretion was investigated in ten patients with hepatic glycogenosis types I and III in order to understand the relationship between hypoglycemia and pancreatic function In all patients both oral glucose tolerance and intravenous arginine infusion tests revealed hypoinsulinemia Decreased urinary C peptide levels with standard food intake also supported dysfunction of pancreatic beta cells On the contrary the normal secretion pattern of glucagon in both types indicated in the arginine loading test intact alpha cells in the pancreas Persistent hypoinsulinism which is apparently an adaptation to hypoglycemia could be an important cause of nutritional dwarfism in both types of glycogenosis The usefulness of the measurement of urinary C peptide which evaluates the pancreatic function and provides management for normal body growth is discussed

KEY WORDS Glycogenosis insulin C peptide glucagon

Hepatic glycogenoses are hereditary inborn errors of carbohydrate metabolism due to glycogenolytic enzyme deficiencies (6) Fasting hypoglycemia is frequently seen in patients with hepatic glycogenosis and it becomes a serious problem affecting the physical development of these patients Therefore it seems valuable to investigate the secretion of insulin and glucagon in this disorder because both hormones are important factors in the maintenance of blood glucose homeostasis

MATERIALS AND METHODS

Ten patients with hepatic glycogenosis seven males and three females aged 1 to 17 years were studied Among them seven cases were of type I (Glucose-6-phosphatase deficiency) and three cases were of type III (Amylo-1-6-glucosidase deficiency) The diagnosis was made on the basis of clinical manifestation and clinical history relevant enzyme assays in leukocytes and liver biopsied specimens (7) and glycogen content in erythrocytes (15)

After overnight fasting either glucose (7 g/kg of body weight 40 g at maximum) was administered orally or a 10% solution of arginine (0.6 g/kg of body weight in 45 min) was administered intravenously In the former test

heparinized blood was withdrawn at 0 30 60 90 and 120 min and in the latter at 5 15 30 45 60 and 90 min Each blood sample 2 ml was placed promptly into a chilled tube containing 7 000 U of Trasylol in a volume of 0.2 ml and the plasma was separated as soon as possible with a refrigerated centrifuge The control group consisted of ten normal healthy children aged between 3 to 10 years All studies were carried out after obtaining informed parental consent

Plasma glucose was measured by the glucose oxidase method (5) plasma immunoreactive insulin (IRI) by the double antibody method of radioimmunoassay (4) and plasma pancreatic immunoreactive glucagon (IRG) by the same method of radioimmunoassay with antiserum 30 K (14)

The urinary C peptide measurement was carried out by the method of Kuzuya et al (8) using freshly collected 24 hour urine samples Urine samples were usually assayed at 1:10 dilutions as Kuzuya et al (8) recommended Four patients with type I glycogenosis and three patients with type III 6-12 years of age were fed 70-80 cal/kg (10) which consisted of 50-60% carbohydrate 20-25% protein and 20-25% fat as Fernandes et al (9) suggested The renal function was intact in all cases of glycogenosis This finding excluded the influence of abnormal renal metabolism on the urinary excretion of C peptide Con

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reticuloendothelial system are reflected in the serum ferritin levels (8). As far as ferritin is concerned we found no difference in these iron stores in infants of smokers and non smokers. This was also the case with regard to the serum iron and the transferrin concentrations in cord serum.

The inversely found between the infants' hematocrit values on the 1st day of life and their cord serum ferritin concentrations (Fig 2) suggests that increased red cell production may cause iron incorporation into erythrocytes at expense of iron storage in the bone marrow and the reticuloendothelial system.

IUGR is often caused by a placental dysfunction syndrome with impaired oxygen supply to the fetus. High hematocrit levels and a transitory postnatal thrombocytopenia frequently occur in these infants (13). The thrombocytopenia seems to be related to chronic intrauterine hypoxia (11) probably because of a competition on common stem cells for erythropoiesis and thrombopoiesis (7). In fetuses suffering from a placental dysfunction syndrome maternal smoking may cause an additional hypoxic stress. This may explain why low thrombocyte counts more frequently occurred in neonates with IUGR of smoking mothers than in IUGR infants of non smokers (13). In normal pregnancy however the hypoxic stress on the fetus from maternal smoking may not exceed the compensatory mechanisms for thrombopoiesis.

ACKNOWLEDGEMENTS

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REFERENCES

- Andrews J. Thiocyanate and smoking in pregnancy. *Br J Obstet Gynaecol* 80: 810 1973.
- Asmussen I. Ultrastructure of the human placenta at term. Observations on placentas from newborn children of smoking and non smoking mothers. *Acta Obstet Gynecol Scand* 56: 119 1977.

- Cole P V, Hawkins L H & Roberts D. Smoking during pregnancy and its effects on the fetus. *Br J Obstet Gynaecol* 79: 787 1972.
- Gunn S M & Shaw H A. Effect of maternal smoking on hemoglobins and hematocrits of the newborn. *Am J Clin Nutr* 31: 557 1978.
- Häggl P & Falkanger B. In vitro assay for erythropoietin. Erythroid colony formation in methylcellulose used for the measurement of erythropoietin in plasma. *Blood* in press.
- Isager H & Hagerup L. Relationship between cigarette smoking and high packed cell volume and hemoglobin levels. *Scand J Haematol* 8: 241 1971.
- Jackson C W & Edwards C C. Biphasic thrombopoietic response to severe hypobaric hypoxia. *Br J Haematol* 35: 233 1977.
- Lanzkowski P. Iron metabolism in the newborn infant. *Clin Endocrinol Metabol* 5: 149 1976.
- Matoth Y & Zaizov R. Effect of intrauterine growth on erythropoiesis. *Exp Hematol* 6: 451 1978.
- Meberg A. Unpublished data.
- Meberg A & Halvorsen S. Transitory thrombocytopenia in small-for-dates infants and in newborn mice exposed to hypobaric hypoxia, cigarette smoke and CO gas inhalation during pregnancy. *Proc Europ Soc Paediatr Res Annual Meeting, Turku* 1978 p 58.
- Meberg A, Sande H, Foss O P & Stenwig J T. Smoking during pregnancy—effects on the fetus and on thiocyanate levels in mother and baby. *Acta Paediatr Scand* in press.
- Meberg A, Ørstavik I & Sovde A. Erythroblastemia and thrombocytopenia in small for gestational age infants. Relation to intrauterine hypoxia, infections and maternal smoking. *Acta Paediatr Belg* 31: 713 1978.
- Pettigrew A R & Fell G S. Simplified colorimetric determination of thiocyanate in biological fluids and its application to investigation of the toxic amblyopias. *Clin Chem* 18: 996 1972.
- Schooley J C & Mahlmann L J. Hypoxia and the initiation of erythropoietin production. *Blood Cells* 1: 429 1975.
- Smith J R & Landaw S A. Smokers' polycythemia. *N Engl J Med* 298: 6 1978.
- Spira A, Fournier R, Grob J C, Dreyfus J & Schwartz D. Smoking during pregnancy and placental blood flow. *Proc 6th Europ Congress of Perinatal Medicine, Vienna* 1978.

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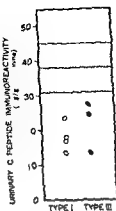


Fig. 3 Urinary C peptide levels are expressed as $\mu\text{g/g}$ creatinine for type I (○) and type III (●) glycogenosis with shaded area as control level of mean \pm standard deviation

taking the recommended food with enough calories

DISCUSSION

Though hormonal function seems important in glycogenoses in which hypoglycemia is one of the most significant symptoms only a few reports were concerned with the study on the secretion of insulin and glucagon in the patients with hepatic glycogenosis. Lockwood et al. (9) reported abnormal glucose tolerance in five patients with type I glycogenosis and concluded that it was due to the insufficient secretion of insulin. Our results also indicated that the function of pancreatic beta cells in types I and III was below normal level in ordinary glucose tolerance test. Moreover we confirmed the insufficient function of beta cells with the direct stimulation with infused arginine. According to Lockwood et al. (9) during the adolescent period the decreased beta cell responsiveness might be an adaptive process and thus alleviate hypoglycemic attack. But because all cases with type I including three cases of early childhood clearly showed hypoinsulinemia the adaptation seems to be completed promptly as early as the early infantile period and does not explain

the clinical stability and improvement of hypoglycemia.

As Horwitz et al. (6) mentioned assay of urinary C peptide is a useful method of integrated measurement of beta cell secretion for a long period. Approximately 5% of C peptide secreted by the beta cells appears in the urine as against only 0.1% of secreted in sulim (6). Although the patients theoretically were given enough calories urinary C peptide levels expressed as $\mu\text{g/g}$ creatinine were significantly lower in both types than in the controls (Fig. 3). These data clearly indicated that the patients with hepatic glycogenosis suffered from chronic and mild deficiency of insulin.

The hypersecretion of glucagon was seen only in type I. In both types of hepatic glycogenosis the pancreatic alpha cells normally reacted to glucose and arginine loading tests. Hence hypoinsulinism may not directly cause the hypersensitivity of pancreatic alpha cells or hyperglucagonemia. Sadeghi-Nejad et al. (13) reported the accelerated recycling process between glycogen and lactate in four patients of type I using a constant infusion of ^{14}C lactate. Thus lactate which is present in increased amounts in the plasma with type I is interpreted as the source of glycogen synthesis for maintaining normoglycemic state. It favors hyperglucagonemia (1). The hormonal characterization in hepatic glycogenoses seems most likely to be limited to hyposecreteness of the pancreatic beta cells and independent of the function of alpha cells of the pancreas.

Insulin is a hormone which generally favors anabolic processes. It increases the availability of glucose for glycogenesis and lipogenesis and stimulates protein synthesis (16). Accordingly if this abnormal hormonal situation in sulim deficiency is prolonged for an extended period of time it may cause the insufficiency of anabolic process and growth retardation in hepatic glycogenosis as in decompensated insulin-deficient diabetic children (11). Roe & Kogut (12) mentioned that the restoration of hypoglycemia caused normalization of the

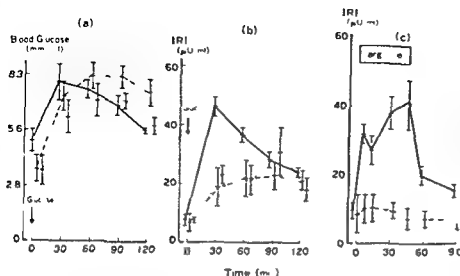


Fig. 1 Response of blood glucose (a) and plasma immunoreactive insulin (IRI) (b) in the oral glucose tolerance test and response of plasma IRI in the arginine infusion test (c). Each bar represents the value of mean \pm standard deviation for the control group (—) type I (---) and type III glycogenosis (....). In Fig. 1c both types I and III are illustrated together (---) since they did not show a different secretion pattern of IRI.

control values were obtained with urine samples from twenty age matched healthy children.

RESULTS

In both types the fasting blood glucose was slightly lower than normal limits (3.7 ± 0.9 mmol/l in type I, 3.5 ± 0.8 mmol/l in type III) and the maximum elevation of blood sugar was obtained between 60 and 90 min after glucose load. The decrease of blood glucose after the peak was very slow as found in diabetes and was still 7.7 ± 0.9 mmol/l at 120 min in type I (Fig. 1a).

The basal value of IRI (9.5 ± 3.3 μ U/ml) was within normal limit. But the maximum value of IRI after the oral glucose loading was less than 70% of that to the controls and its reaction was considerably delayed. The highest value was obtained mostly at later than 60 min (Fig. 1b). Here the straight relationship between blood glucose and IRI was shown.

On the other hand before and after the arginine load the value of plasma IRG was generally high in type I although in type III it was within the control range (68 ± 8 pg/ml) (Fig. 2). Normal suppressive reaction of IRG after the oral glucose load was seen in all cases (data not shown).

Blood glucose was not changed with arginine loading in either type of hepatic glycogenosis. IRI level of all patients was low and

two phase secretion was not detected (Fig. 1c). Quick response of IRG to arginine infusion was observed within 5 min in all cases (Fig. 2).

Fig. 3 shows urinary C peptide content in patients with glycogenosis and control subjects. Patients with both types of glycogenosis excreted smaller amount of daily C peptide expressed as μ g/g creatinine while they were

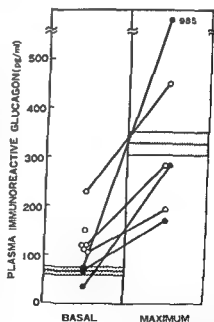


Fig. 2 Plasma immunoreactive glucagon (IRG) levels at basal condition (left) and at maximum secretion after the arginine loading (right) are shown for type I glycogenosis (O) and type III glycogenosis (●). Shaded area is mean \pm standard deviation of control value.

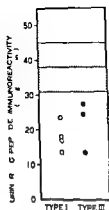


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Insulin is a hormone which generally favors anabolic processes. It increases the availability of glucose for glycogenesis and lipogenesis and stimulates protein synthesis (16). Accordingly if this abnormal hormonal situation in insulin deficiency is prolonged for an extended period of time it may cause the insufficiency of anabolic process and growth retardation in hepatic glycogenosis as in decompensated insulin deficient diabetic children (11). Roe & Kogut (12) mentioned that the restoration of hypoglycemia caused normalization of the

concentration of insulin and glucagon in serum. But our results with oral glucose tolerance tests (Fig. 1a, b) indicated that the normalization of blood insulin level might need some longer preparative time because hyperglycemia after a single oral glucose loading brought only a slight increase of the serum insulin level. Therefore the prolonged nocturnal intragastric infusion of glucose proposed by Greene et al. (3) seems to be one of the best treatments for hepatic glycogenoses in order to restore the imbalance of hormones. But the accurate clinical index to an adequate carbohydrate administration to the patients is not available at present. The measurement of urinary C-peptide may be a reliable index to maintain normal body growth of patients with hepatic glycogenosis.

REFERENCES

1. Exton J. H. & Park C. R. Control of gluconeogenesis in liver. II. Effects of glucagon, catecholamines and adenosine 3',5'-monophosphate on gluconeogenesis in the perfused rat liver. *J Biol Chem* 243: 4189, 1968.
2. Fernandes J. & Pikaar N. A. Hyperlipemia in children with glycogen storage disease. *Am J Clin Nutr* 22: 617, 1969.
3. Greene H. L., Slonim A. E., O'Neill J. A. Jr. & Burr I. M. Continuous nocturnal intragastric feeding for management of type I glycogen storage disease. *N Engl J Med* 294: 423, 1976.
4. Hales C. N. & Randle P. J. Immunoassay of insulin with antibody precipitate. *Biochem J* 88: 137, 1963.
5. Hill J. H. & Kessler G. An automated determination of glucose utilizing a glucose oxidase peroxidase system. *J Lab Clin Med* 57: 970, 1961.
6. Horwitz D. L., Rubenstein A. H., Mako M. E., Cruz A. & Bliv P. M. C-peptide in conditions other than diabetes mellitus. *Diabetes* 27: 767, 1978.
7. Howell R. R. The glycogen storage disease. In J. H. Stanbury, J. B. Wyngaarden & D. S. Fredrickson (eds.), *The metabolic basis of inherited disease*. McGraw Hill, New York, 1978, pp. 137-159.
8. Kuzuya T., Matsuda A., Sakamoto Y., Tanabashi S. & Kajinuma H. C-peptide immunoreactivity (CPR) in urine. *Diabetes* 27: 210, 1978.
9. Lockwood D. H., Merimee T. J., Edgar P. J., Greene M. L., Fujimoto W. Y., Seegmiller J. E. & Howell R. R. Insulin secretion in type I glycogen storage disease. *Diabetes* 18: 755, 1969.
10. National Nutritional Committee. *Nutritional demand for Japanese and its explanation*. Daichi Shuppan, Tokyo, 1975, pp. 23-48 (in Japanese).
11. Petersen H. D., Korgaard B., Deckert T. & Nielsen E. Growth, body weight and insulin requirement in diabetic children. *Acta Paediatr Scand* 67: 453, 1978.
12. Roe T. F. & Kogut M. D. The pathogenesis of hyperuricemia in glycogen storage disease, type I. *J Pediatr Res* 11: 664, 1977.
13. Sadeghi-Nejad A., Presente E., Binkiewicz A. & Senior B. Studies in type I glycogenosis of the liver—the genesis and disposition of lactate. *J Pediatr* 85: 49, 1974.
14. Sakurai H. & Imura H. A sensitive radioimmunoassay for glucagon using talc method. *Jap J Nucl Med* 10: 135, 1973.
15. Sidbury J. B., Cornblath M., Fisher J. & House E. Glycogen in erythrocytes of patients with glycogen storage disease. *Pediatrics* 27: 103, 1961.
16. Tepperman J. *Metabolic and endocrine physiology*. Year Book Medical Publishers, Chicago, 1973, 3rd ed, pp. 167-198.

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SENSORY NERVE CONDUCTION VELOCITY AND VIBRATORY SENSIBILITY IN JUVENILE DIABETICS

Relationship to Endogenous Insulin

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ABSTRACT Ludvigsson J Johannessen G Heding L Hager A and Larsson Y (Departments of Paediatrics and Neurophysiology University Hospital Linköping Sweden and Novo Research Institute Bagsvaerd Denmark) Sensory nerve conduction velocity and vibratory sensibility in juvenile diabetes Relationship to endogenous insulin Acta Paediatr Scand 68 739 1979 —Sensory nerve conduction velocity (NCV) and the vibratory sense (biothesiometry) were determined in 67 children and adolescents with insulin dependent diabetes Age at onset of diabetes varied between 1-14 years (mean \pm SD 6.5 ± 3.6) and the duration of diabetes between 4-17 years (7.7 ± 3.4) Within ± 3 months of the nerve function tests blood was drawn for determination of C peptide and insulin antibodies (IgG and IRI) A low NCV (< 50 m/s) in the sural nerve and/or an abnormal vibratory sense (≥ 1.0 microns) were found in 34 patients (50.7%) Measurable fasting serum C peptide $0.04-0.60$ pmol/ml (0.17 ± 0.15) was found in 18 patients (23.9%) All but one patients had insulin antibodies with IgG $0.130-11.029$ mU/ml (2.957 ± 2.509) and total IRI $10-9.120$ μ U/ml (1204 ± 1723) In multiple regression analysis we did not find any correlation between nerve function and sex age or age at onset of diabetes and there was only a weak relationship between NCV and duration However there was a positive correlation between NCV and C peptide ($p < 0.001$) Vibration sense was also better among patients with C peptide ($p < 0.05$) The results support the view that insulin deficiency contributes to peripheral diabetic neuropathy

KEY WORDS Sensory nerve conduction velocity vibratory sense C peptide insulin antibodies juvenile diabetes mellitus

Although neuropathy is said to be one of the most common of diabetic complications (8) little is known about its etiology Several hypotheses have been proposed and many clinical and experimental studies have been done on the subject However most such studies have been done on adults in which it may have been difficult to define onset of diabetes and where non diabetic factors interfere with the evaluation of purely diabetic factors such as insulin deficiency or occurrence of insulin antibodies

Clinical neuropathy is neither common nor pronounced in children with diabetes but neurophysiological studies of sensory function may reveal impairment of peripheral nerve function even in patients without neurological symptoms and signs (9-10, 13-14)

The aim of this study was to study the occurrence of abnormal sensory nerve function among children and adolescents with diabetes and especially to analyze whether a normal function is associated with a preserved beta cell function

MATERIAL

The patients consisted of 67 children and adolescents aged 5-20 years 38 males and 29 females Age at onset of diabetes varied between 1-14 years (mean \pm SD 6.4 ± 3.6) and the duration of diabetes between 4-17 years (7.7 ± 3.4) All patients were insulin dependent and were treated with insulin 1-2 times daily regular physical exercise and an optimal diabetic diet All patients except two tested their urine 4 times every day by the Clinitest method They noted the results in a diary which was shown to the doctor at the diabetic clinic 4-8 times a year Further characteristics of the material have been described in detail in previous publications (2*)

Table 1 Distribution of sensory nerve conduction velocity (NCV) amplitude of action potential and biothesiometry in 67 juvenile diabetics

	N suralis		N medianus		N ulnaris	
	Left	Right	Left	Right	Left	Right
NCV						
<45 m/s	3	3	6	6	0	1
45-49	16	17	4	8	5	4
50-55	24	22	18	20	11	20
>55	17	19	37	30	49	41
Mean	52.4	52.2	55.7	54.3	58.5	57.4
S D	6.1	5.6	6.3	6.2	5.8	5.7
Range	34-69	37-63	41-69	39-71	46-72	44-69
Amplitude						
<10	38	39	7	9	19	24
10-20	18	18	35	30	38	35
>20	4	4	23	26	6	6
Mean	8.7	8.8	19.5	18.1	12.2	11.7
S D	5.6	5.2	9.3	8.9	5.1	5.1
Range	1-25	1.5-20.9	3.6-41.0	1.7-45	1.5-25	1.7-27.5
Biothesiometry						
<0.5 microns	15	14	19	15	17	17
0.5-0.99	26	31	30	38	32	38
≥1.0	14	13	8	6	9	5
Mean	0.80	0.76	0.66	0.62	0.60	0.67
S D	0.63	0.50	0.43	0.25	0.25	0.76
Range	0.25-3.70	0.25-2.90	0.30-3.2	0.20-1.05	0.2-1.2	0.30-1.40

METHODS

In the measurement of the sensory nerve conduction velocity (NCV) a digital signal averager (Biomac 1000) was used. The stimulus duration used was 0.1 msec. Spring loaded electrodes (15) were used for stimulation of the third finger (median nerve) and the fifth finger (ulnar nerve). The sural nerve was stimulated behind and below the lateral malleolus using circular chlorided silver electrodes. The hands and the lower extremities were warmed when necessary using hot water bottles or, sometimes immersion in warm water. The skin temperature near the nerves was kept in the range 30-35°C in the hands and 30-40°C in the lower extremities.

A supramaximal stimulation was used. The sensory nerve action potentials were registered with conventional bipolar technique. The signals were preamplified to the digital averager. The averaged signals were written out on an X-Y recorder whereafter the latency from the onset of the stimulus to the beginning of the first negative deflection could be measured manually as well as the peak to peak amplitude of the action potential.

For the measurements of vibratory thresholds a biothesiometer (Ohio) was used. The measurements were made on the tip of the third and fifth finger (median and ulnar nerves) and on the fifth toe (sural nerve). The biothesiometer is constructed so as to produce vibrations of a plastic tip with the double frequency of the domestic alternating current which in Sweden means 120 c/s (2×60 c/s). The vibrator was placed against the finger tip or the toe with an even pressure. The patient was told to

report at the moment when he began to perceive the vibration while the voltage was slowly increased and to report the disappearance of the vibratory sensation as the voltage was decreased. Several measurements were made in each case and mean values calculated from measurements for each individual finger or toe.

On some occasion within ±3 months of the nerve function test blood was drawn from the fasting patients prior to their morning insulin injection. Serum was stored at -18°C. The binding of insulin to IgG was determined by the method of Christiansen (5) and total immunoreactive insulin (IRI) and serum C peptide according to Heding (19, 20).

RESULTS

Clinical signs of neuropathy were found in 15 patients (22.4%) (mostly absence of deep tendon reflexes) on some occasion over a period of three years. However only two of these patients experienced subjective symptoms. In 12 of the 15 patients there was a low NCV (<50 m/s) in the sural nerve and/or abnormal vibratory sense measured by the biothesiometer (≥1.0 microns). Altogether 34 patients (50.7%) showed such abnormal neurophysiological values thus including 22 patients

Table 2 Correlation between left and right side for each nerve regarding nerve conduction velocity amplitude biothesiometry

	NCV		AMP		BIO	
	r	p	r	p	r	p
Suralis	+0.81	<0.001	0.71	<0.001	0.80	<0.001
Ulnaris	0.71	<0.001	0.66	<0.001	0.76	<0.001
Medianus	0.83	<0.001	0.65	<0.001	0.80	<0.001

without any clinical signs. Table 1 shows the distribution of NCV amplitudes and biothesiometric values for the three nerves. NCV and amplitude were lower and biothesiometry values higher for *n. suralis* than for *n. medianus* and *n. ulnaris*. There was a good correlation between the right and left sides of the respective nerves regarding both NCV amplitude and biothesiometry (Table 2) and the correlations for NCV between the different nerves were also rather high (*sul/uln* $r=0.66$, $p<0.001$; *sul/med* $r=0.45$, $p<0.001$; *uln/med* $r=0.65$, $p<0.001$). The correlation between NCV and amplitude was weakly positive at highest in medianus ($r=0.38$, $p<0.01$) and there was also a relationship between NCV and biothesiometric values in each single nerve (*n. suralis* $r=0.34$, $p<0.02$) and between biothesiometry and amplitude (*n. suralis* $r=0.46$, $p<0.001$). The correlation between NCV and skin temperature was positive (*n. suralis* $r=0.45$, $p<0.001$).

A good or acceptable metabolic balance according to the number of urine tests without glucosuria in percentage of all tests performed (Table 3) was found in 53 patients (81.5%) out of those 65 who tested their urine regularly.

Measurable C-peptide was found in 16 patients (23.9%) between 0.04–0.60 pmol/ml (0.17 ± 0.15). All patients except one had insulin antibodies with IgG 0.130–11.029 (2.957 ± 2.091) mU/ml and total IRI 10.9–120 (1.204 ± 1.723) μ U/ml.

In multiple regression analysis an effort was made to express NCV and biothesiometry as a function of several interdependent variables. When skin temperature was held constant it

was evident that sex and age at onset of diabetes played no role and even duration of diabetes was only weakly negatively correlated to NCV ($n.s.$). Neither did we find any significant relationship to glucosuria. However, there was a negative correlation between amount of insulin antibodies (IgG) and NCV ($p<0.01$) while the correlation between NCV and measurable fasting serum C-peptide (>0.04 pmol/ml) was significantly positive ($p<0.001$). Vibration sense evaluated by biothesiometry was not correlated to insulin antibodies but it was significantly better in those of lower age ($p<0.05$) and in patients with measurable C-peptide ($p<0.05$).

DISCUSSION

From studies mainly on motor NCV several authors have reported peripheral neuropathy among diabetic children without any clinical symptoms or signs on routine neurological examination (6, 9, 13, 14). We studied vibratory sense and sensory NCV as sensory nerve fibres seem to be affected before motor nerves (17, 25). Our definition of low NCV as <50 m/s and abnormal vibratory sense as ≥ 1.0

Table 3 Diabetic control

Index = incidence of urine tests with less than 1% glucose

Index (%)	n	%
≥ 90	6	9.2
70–89	22	33.8
50–69	25	38.5
<50	12	18.5
$x=67.6$	65	100.0

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S D	6.1	5.6	6.3	6.2	5.8	5.7
Range	34-69	37-63	41-69	39-71	46-72	44-69
Amplitude						
<10	38	39	7	9	19	24
10-20	18	18	35	30	34	35
>20	4	4	23	26	11	6
Mean	8.7	8.8	19.5	18.1	12.2	11.7
S D	5.6	5.2	9.3	8.9	5.1	5.1
Range	1-25	1.5-20.9	3.6-41.0	1.7-45	1.5-25	1.7-33.5
Biothesiometry						
<0.5 microns	15	14	19	15	17	17
0.5-0.99	26	31	30	19	32	38
≥1.0	14	13	8	6	9	5
Mean	0.80	0.76	0.66	0.62	0.60	0.62
S D	0.63	0.50	0.43	0.25	0.25	0.26
Range	0.25-3.70	0.25-2.90	0.30-3.2	0.20-1.05	0.2-1.2	0.30-1.40

METHODS

In the measurement of the sensory nerve conduction velocity (NCV) a digital signal averager (Biomac 1000) was used. The stimulus duration used was 0.1 msec. Spring loaded electrodes (15) were used for stimulation of the third finger (median nerve) and the fifth finger (ulnar nerve). The sural nerve was stimulated behind and below the lateral malleolus using circular chlorided silver electrodes. The hands and the lower extremities were warmed when necessary using hot water bottles or sometimes immersion in warm water. The skin temperature near the nerves was kept in the range 30-35°C in the hands and 30-40°C in the lower extremities.

A supramaximal stimulation was used. The sensory nerve action potentials were registered with conventional bipolar technique. The signals were preamplified to the digital averager. The averaged signals were written out on an X-Y recorder whereafter the latency from the onset of the stimulus to the beginning of the first negative deflection could be measured manually as well as the peak to peak amplitude of the action potential.

For the measurements of vibratory thresholds a biothesiometer (Ohio) was used. The measurements were made on the tip of the third and fifth finger (median and ulnar nerves) and on the fifth toe (sural nerve). The biothesiometer is constructed so as to produce vibrations of a plastic tip with the double frequency of the domestic alternating current which in Sweden means 120 c/s (2×60 c/s). The vibrator was placed against the finger tip or the toe with an even pressure. The patient was told to

report at the moment when he began to perceive the vibration while the voltage was slowly increased and to report the disappearance of the vibratory sensation as the voltage was decreased. Several measurements were made in each case and mean values calculated from measurements for each individual finger or toe.

On some occasion within ±3 months of the nerve function test blood was drawn from the fasting patients prior to their morning insulin injection. Serum was stored at -18°C. The binding of insulin to IgG was determined by the method of Christiansen (5) and total immunoreactive insulin (IRI) and serum C peptide according to Heding (19, 20).

RESULTS

Clinical signs of neuropathy were found in 15 patients (22.4%) (mostly absence of deep tendon reflexes) on some occasion over a period of three years. However only two of these patients experienced subjective symptoms. In 12 of the 15 patients there was a low NCV (<50 m/s) in the sural nerve and/or abnormal vibratory sense measured by the biothesiometer (≥1.0 microns). Altogether 34 patients (50.7%) showed such abnormal neurophysiological values thus including 22 patients

- normic nerve function in diabetic ketoacidosis *Lancet* **II** 167 1967
- 4 Chochinov R H Ulliyot L E & Moorhouse J A Sensory perception thresholds in patients with juvenile diabetes and their close relatives *N Engl J Med* **286** 1733 1977
 - 5 Christiansen A H Radioimmuno-electrophoresis in the determination of insulin binding to IgG Methodological studies *Horm Metab Res* **5** 147 1973
 - 6 Eeg-Olofsson O & Petersén O Childhood diabetic neuropathy Clinical and neurophysiological study *Acta Paediatr Scand* **55** 163 1966
 - 7 Eliasson S G Nerve conduction changes in experimental diabetes *J Clin Invest* **43** 2353 1964
 - 8 Ellenberg M Diabetic neuropathy In J S Bajaj (ed.) *Insulin and metabolism* Excerpta Medica Amsterdam 1977 p 109
 - 9 Eng G D Hung N & August G P Nerve conduction velocity determination in juvenile diabetes *Diabetes in Juveniles Mod Probl Paediatr* **12** 213 Karger Basel 1975
 - 10 Fagerberg S E Diabetic neuropathy *Acta Med Scand* **164** Suppl 345 1959
 - 11 Fagerberg S E Petersén I & Walhelmsen S G Motor disturbances in diabetes mellitus A clinical study using electromyography and nerve conduction velocity determination *Acta Med Scand* **174** 711 1963
 - 12 Gabbay K H Hyperglycemia polyol metabolism and complications of diabetes mellitus *Ann Rev Med* **26** 571 1975
 - 13 Gamstorp I Conduction velocity of peripheral motor nerves in mental retardation diabetes and various neurological diseases in childhood *Acta Paediatr Scand* **53** 408 1964
 - 14 Gamstorp I Shelburne S A Engleson G Redondo D & Trausman H S Peripheral neuropathy in juvenile diabetes *Diabetes* **15** 411 1966
 - 15 Goldberg M J Rogoff J B & Levine B Spring loaded electrode for sensory nerve conduction *Arch Phys Med Rehab* **49** 665 1968
 - 16 Gregersen G Diabetic neuropathy influence of age sex metabolic control and duration of diabetes on motor conduction velocity *Neurology (Minneapolis)* **17** 977 1967
 - 17 Gregersen G Vibratory perception thresholds and motor conduction velocity in diabetics and non-diabetics *Acta Med Scand* **183** 61 1968
 - 18 Green D A De Jesus P V Jr & Winegrad A I Effects of insulin and dietary myoinositol on impaired peripheral motor nerve conduction velocity in acute streptozotocin diabetes *J Clin Invest* **55** 13 6 1975
 - 19 Heding L G Determination of total serum insulin (IRI) in insulin treated diabetic patients *Diabetologia* **8** 260 1972
 - 20 Heding L G Radioimmunological determination of human C peptide in serum *Diabetologia* **11** 541 1975
 - 21 Kazibutowska A Polyneuropathy and motor fibre conduction velocity in children and adolescents with diabetes *Neur Neurochir Pol* **8** 519 1974
 - 22 Ludvigsson J *Metabolic control in juvenile diabetes mellitus* Linköping University Medical Dissertation No 42 Zlataryckerna Linköping 1976
 - 23 Ludvigsson J & Heding L G Beta-cell function in children with diabetes *Diabetes* **27** Suppl 1 230 1978
 - 24 Marcus J Ehrlich E Kelly M & Murphy E G Nerve conduction in childhood diabetes *CMAJ* **108** 1116 1973
 - 25 La Montagne A & Buchthal F Electrophysiological studies in diabetic neuropathy *J Neurol Neurosurg Psychiatr* **33** 447 1970
 - 26 Schlaepfer W W Gerritsen G C & Dulin W E Segmental demyelination in the distal peripheral nerves of chronically diabetic Chinese hamsters *Diabetologia* **10** 541 1974
 - 27 Schmidt R E Matschinsky F M & Godfrey D A Fast and slow axoplasmic flow in sciatic nerve of diabetic rats *Diabetes* **24** 1081 1975
 - 28 Thomas P K & Eliasson S (In P J Dyck P K Thomas & E H Lamberg (eds)) *Diabetic neuropathy peripheral neuropathy* WB Saunders Philadelphia 1975 vol 2 p 956
 - 29 Ward J D Structure and biochemistry of degenerative lesions in diabetes mellitus Pathogenesis of diabetic neuropathy *Proceedings of the 8th Congress of the International Diabetes Federation* (ed W J Malaisse et al) Excerpta Medica Amsterdam 1974 p 419
 - 30 Winegrad A I & Greene D A Diabetic polyneuropathy The importance of insulin deficiency hyperglycemia and alterations in myoinositol metabolism in its pathogenesis *N Engl J Med* **295** 1416 1976

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microns of motion is chosen on the basis of the reports from other investigators (2-4) as well as from the results in non diabetics of our own laboratory. We can conclude that many diabetic children without clinical symptoms and signs seemed to have abnormal sensory nerve function especially in the lower extremities. This confirms previous clinical experience. Already at the age of 7 years abnormal values were seen in some cases after a duration of only 4 years.

The cause or causes of diabetic neuropathy are unknown. Diabetic microangiopathy is a major cause (10) and probably be excluded as neurological disturbances are found very early after onset when no vascular complications other than possibly functional seem to exist (4). Most workers believe that the development of peripheral neuropathy is conditioned by some metabolic disturbance (8, 28, 29). There seems to be derangements both of the polyol pathway (12) and the myoinositol glucose and lipid metabolism (1). Eliaeson (7) observed that in rats that were made hyperglycemic sensory NCV was impaired. It has been shown that insulin treatment can prevent this impaired function only when it prevented prolonged periods of hyperglycemia (18, 30). In rats acute diabetes causes decreased axonal transport. These alterations can be reversed by insulin treatment (27). Furthermore it has been shown that in nerves with impaired motor NCV the concentration of free myoinositol is reduced which appears to be a consequence of insulin deficiency or hyperglycemia or both (30). Peripheral nerve lesions similar to those found in patients with overt diabetic polyneuropathy have been found in Chinese hamsters with spontaneous diabetes related to the duration and severity of hyperglycemia (26). Thus insulin deficiency and hyperglycemia seem to play primary roles in the pathogenesis of impaired nerve function in experimental animals with acute diabetes.

The relevance of observations in experimental animals to juvenile diabetes in children cannot be assessed without clinical studies.

Other authors have reported a relation between disturbed nerve function and long duration of juvenile diabetes (9, 13, 14) and possibly poor metabolic control (6, 14). Disturbed peripheral nerve function has been found during ketoacidosis (3). We have been able to measure the residual endogenous insulin secretion which better than conventional treatment protects the patients against insulin deficiency and thus contributes to less ketoacidosis and better metabolic balance (23). When taking several factors into consideration simultaneously in the multiple regression analysis we found that age was of no importance neither age at onset nor duration of diabetes in itself while absence of C peptide which often but not always is seen after long duration and high amount of insulin antibodies were significantly negatively correlated to sensory NCV. The correlation between residual insulin secretion and nerve function was also confirmed when biothesiometry was studied but insulin antibodies showed no such relationship. As there is a negative correlation between C peptide and insulin antibodies (??) the negative relationship between insulin antibodies and NCV could be secondary to the association between C peptide and NCV. Thus the role of insulin antibodies is uncertain. However our results do support the view that a major cause of peripheral diabetic neuropathy is insulin deficiency.

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REFERENCES

1. Anderson J W. Metabolic abnormalities contributing to diabetic complications. II. Peripheral nerves. *Am J Clin Nutr* 29: 402, 1976.
2. Buchthal F & Rosenfalck A. Evoked action potentials and conduction velocity in human sensory nerves. *Brain Res* 3: 1, 1966.
3. Campbell I W, Ewing D J, Harrower A B D, Neilson J M M, Fraser D M, Baldwin V S, Murray A & Clarke B F. Peripheral and auto-

ON THE EPIDEMIOLOGY OF HUMAN TOXOPLASMOSIS IN SCANDINAVIA ESPECIALLY IN CHILDREN

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ABSTRACT Hult G Lagercrantz R and Shee P R (Swedish National Bacteriological Laboratory Department of Paediatrics Karolinska Hospital Stockholm Sweden and Department of Epidemiology Upstate University Syracuse NY USA) On the epidemiology of human toxoplasmosis in Scandinavia especially in children Acta Paediatr Scand 68 745 1979 —A random sample representing approx one third of pre school children and approx 95% of school children in age groups 8 11 13 and 15 years in a suburb of Stockholm were examined for antibodies to Toxoplasma Antibodies were uncommon in small children They gradually appeared throughout childhood but especially in adolescence and adult life Antibodies were more common in females than in males Even small girls were more often infected than boys of corresponding age The difference becomes marked in puberty and is significant in adults A family study revealed evidence of familial aggregation of the infection albeit with borderline significance No correlation was found between occurrence of antibodies and the presence of cats in the families or reported consumption of raw meat The majority of representative samples of Lapps and Skolt Lapps in northern Scandinavia lacked antibodies to Toxoplasma

KEY WORDS Toxoplasma epidemiology family study

There is much about toxoplasmosis to excite all our fancies whether we be pediatricians internists ophthalmologists serologists parasitologists or shoe leather epidemiologists (Feldman 1960)

The toxoplasma parasite infects many animal species including man Infection is most often subclinical The infection rate in man varies considerably from country to country From 5 to 75% of the adult populations in different parts of the world are or have been infected (1-4 6-8 17)

The source of human infection was for a long time unknown However early observations indicated that toxoplasmosis may be transmitted by infected meat (12 18) and this was later confirmed by Desmonts et al (3) In 1969 Kean et al (13) could attribute a small epidemic of toxoplasmosis to the ingestion of hamburgers The role of the cat in the transmission of toxoplasmosis was first indicated by Hutchinson (10) who could demonstrate

that cat faeces can be infective This observation led to detailed studies by several groups resulting in the present views that toxoplasma is a coccidia like parasite with the cat as final host and that the infective stage in the cat faeces is the oocyst (4 5 11 13 20)

Thus two routes of infection have been demonstrated It is however far from clear to what extent each of them contributes to human disease Nor do we know whether there may also be other ways by which man can be infected

We have studied the occurrence of toxoplasma antibodies in three populations 1 Children and adults in Sundbyberg a suburb of Stockholm Sweden 2 Skolt Lapps in northern Finland 3 Lapps in northern Norway We want to compare the incidence of the disease in these different populations and to elucidate the role of age sex domestic animals and family contact

Table 1 Age and sex adjusted prevalences (%) of positive titers in families of positive and negative proposition by family size

Family size	Persons (no.)	Index		Difference
		Positive	Negative	
1	13	46	46	0
2	138	48	38	10
3	85	41	35	6
4	88	47	27	15
5	30	77	40	-13
Total	544	4	35	7

those of index negative ones as to age, sex and family size. The age and sex differences between families of the positive and negative proposition are very slight (Table 1). Of critical concern in this analysis are the distributions of the other family members.

In families of sizes 2, 3 or 4 which include the great majority of non index participants the age and sex adjusted prevalences were higher in the positive index children's families by from 6 to 15%. The grand mean difference of 7% was obtained as the inverse variance weighted average of the differences for the 5 family size groupings. The test of significance is based on the standard error of the weighted mean determined from interfamily variability rather than individual variability. The 7% excess in the age, sex and family size adjusted prevalence of positive individuals in families of positive index members was significant at the $p < 0.06$ two-tailed level. This is borderline since it does not quite reach the customary $p < 0.05$ two-tailed level of significance.

The geometric mean titers (GMT) of those who were positive in the families of positive proposition was 51 while for those in families of negative proposition it was 45. Thus the ratio is 1.13:1 for the families of positive versus negative index children. After adjustment for age and sex the ratio is 1.18:1 not significantly different from a ratio of 1:1 ($p < 0.26$). (In the test of significance age and sex are taken into

account and families rather than individuals are the analytical units.)

While the excess prevalence of positivity in families of positive index children was of borderline significance ($p < 0.06$) the excess GMT although not in itself significant ($p < 0.26$) is supportive. Thus in comparing the GMT's of all family participants excluding proposition there was a significant excess GMT in the families of positive proposition ($p < 0.04$). This is evidence albeit with borderline significance of familial aggregation.

Study of Lapps and Skolt Lapps

Antibody titers in different ethnic groups in northern Norway and Finland are presented in Table 2. In comparison with Sundbyberg there were very few infected individuals in the populations of Lapps and Skolt Lapps in the North.

In contrast to the Sundbyberg families the Lappish populations live isolated. This is true particularly for the Skolt Lapps who live in groups of two or three families within a large area. Most of the houses are situated at a considerable distance from roads and the Lapps have to walk or ski to the nearest community. The Lappish families live in close contact with their domestic animals. By tradition all families have one or several dogs but also cats.

Table 2 Prevalence of toxoplasma antibodies in Lapps and Skolt Lapps according to sex and age

Age	Females		Males	
	Total	DT pos	Total	DT pos
<i>Lapps</i>				
0-10	34	-	38	-
11-20	46	1	64	-
21-60	160	5	130	5
Sum	240	6 (2.5%)	232	5 (2.1%)
<i>Skolt Lapps</i>				
0-10	8	-	78	1
11-20	40	-	60	4
21-60	57	6	63	2
Sum	125	6 (4.8%)	151	7 (4.6%)

MATERIAL AND METHODS

The study in Sundbyberg

Sundbyberg is a suburb of Stockholm. Most inhabitants live in apartment houses with a good standard of living. There are no slums. All children are born in hospitals, most infants and toddlers attend well baby clinics, around 20% are in nursery schools. School starts at 7 years of age and is compulsory for 9 years.

A random sample representing approximately one third of families with pre school children and all families with school children in grades 2 (age 8-9 years), 5 (age 11-12 years), 7 (age 13-14 years) and 9 (age 15-16 years) were given a questionnaire concerning the health and development of the children and the occurrence of lymphadenopathy and eye symptoms. The families were also asked if they had any domestic animal at home. Five per cent of the families did not answer the questionnaire and did not participate in other parts of the study.

Family study. Parents to children who in the first study had dye test antibody titers of 250 or higher (positive index children) and parents to children with no demonstrable antibodies (negative index children) were contacted. Altogether 554 individuals belonging to 205 families were examined. Parents were interviewed (by G H and R L) regarding their own and their children's health, signs of lymphadenopathy and eye diseases and habits of eating raw or inadequately prepared meat. The children were examined by G H and R L.

Study of Lapps and Skolt Lapps in northern Finland and Lapps in northern Norway

As part of an epidemiological study on tuberculosis 1548 adult Lapps (≥ 15 years) in Kautokeino in northern Norway were screened. Around a third of these individuals were examined for antibodies to echinococcus (9) and toxoplasma.

In still another study 573 Lapps and 176 Skolt Lapps in Inari, northern Finland, were also examined for these antibodies.

Serological tests. Children were bled and sera stored at -70°C until tested in the toxoplasma dye test (DT) and complement fixation test at the National Bacteriological Laboratory, Stockholm.

RESULTS

Antibody and age. The incidence of elevated antibody titers ($\text{DT} \geq 10$ and ≥ 250 respectively) in boys and girls of different ages in Sundbyberg are presented in Fig 1. Data are based on results in a third of the pre school population (probably representing a random sample) and around 95% of the school children. Children below 1 year of age were not examined because their antibodies are most often passively acquired. The adults are the parents to the index children in the family study.

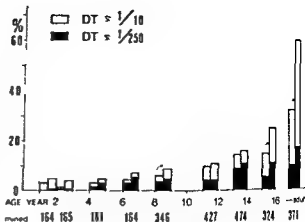


Fig 1 Frequency of positive dye test (DT) in children and adults in Sundbyberg related to age and sex.

Antibodies to toxoplasma are uncommon in small children (Fig 1). They gradually appear, especially in late childhood and adolescence. In the 9th grade approximately 19% of the girls and 14% of the boys were infected. Infection is, however, most often not acquired until adult life.

Antibody and sex. Antibodies were more common in females than in males (Fig 1). Even small girls were more often infected than boys of corresponding age. The difference becomes marked in puberty and is significant in adults ($p < 0.01$).

Antibodies and lymphadenopathy or eye symptoms. Children with lymphadenopathy or eye symptoms did not differ serologically from the others.

Antibodies and pets in the family. Around 5% of the families had cats and 7% dogs. 4% had birds (mostly canaries) and 5% other pets. Families living with these animals did not differ in antibody titer from families without pets.

Antibodies and the consumption of raw meat. Around 10% of the families reported consumption of raw meat (mostly pork) by some or all family members. These families did not differ in infection rate from others. The quantities consumed are not known.

Family study

We did not as planned succeed in matching families to index positive children against

- 4 No correlation between presence of anti bodies and the exposure to cats or the eating of raw meat could be demonstrated
- 5 Toxoplasmosis is quite rare in Lapps and Skolt Lapps in northern Finland and Norway. The reason for this could not be established

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REFERENCES

- 1 Beverley J K, Fleck D G, Kwantes W & Ludlam G B. Age-sex distribution of various diseases with particular reference to toxoplasmic lymphadenopathy. *J Hyg (Camb)* 76: 15, 1976.
- 2 Christie E, Dubey J P & Pappas P W. Prevalence of *Sarcocystis* infection and other intestinal parasites in cats from a humane shelter in Ohio. *J Am Vet Med Assoc* 168: 471, 1976.
- 3 Desmonts G, Couvreur J, Ahson F, Baudelot J & Lelong M. Etude épidémiologique sur la toxoplasmose de l'influence de la cuisson de viandes de boucherie sur la fréquence de l'infection humaine. *Rev Fr Etud Clin Biol* 208: 1007, 1969.
- 4 Dubey J P, Miller N L & Frenkel J K. The *Toxoplasma gondii* oocyst from cat feces. *J Exp Med* 133: 636, 1970.
- 5 Dubey J P & Frenkel J K. Cyst induced toxoplasmosis in cats. *J Protozool* 19: 155, 1972.
- 6 Feldman H A. Epidemiological aspects of toxoplasmosis. In *Human Toxoplasmosis* pp 169-174. Munksgaard, Copenhagen, 1960.
- 7 Harnbræus L. Occurrence of antibodies against toxoplasma and tularensis in a country of Northern Sweden. *Communication 2nd Int Symp Circumpol Health Oulu* 1971: p 48.
- 8 Hedenström G, Huidt G & Lagercrantz R. Toxoplasmosis in children. *Acta Paediatr Scand* 50: 304, 1961.
- 9 Huidt G, Johansson S G O & Lantto S. Echinococcosis in Northern Scandinavia. Immune reactions in Kautokeino Lapps. *Arch Environ Health* 26: 36, 1973.
- 10 Hutchinson W M. Experimental transmission of toxoplasma gondii. *Nature (Lond)* 206: 961, 1965.
- 11 Hutchinson W M, Dunachie F, Sum J C & Work K. Coccidian like nature of *Toxoplasma gondii*. *Br Med J* 1: 143, 1970.
- 12 Jacobs L, Remington J S & Melton M L. A survey of meat samples from swine, cattle and sheep for the presence of encysted toxoplasma. *J Parasitol* 46: 23, 1960.
- 13 Kean H H, Kimball A C L & Christenson W N. An epidemic of acute toxoplasmosis. *JAMA* 208: 1007, 1969.
- 14 Korhonen P & Räsänen S. Occurrence of toxoplasmosis in wild and domestic animals in Northern Finland. *Communication 2nd Int Symp Circumpol Health Oulu* 1971: p 48.
- 15 Kouba K, Stehlikova J, Rajlichova J, Ubner H & Svandova E. Evaluation of some epidemiological factors in human toxoplasmosis. *Z Gesamte Inn Med* 31: 583, 1976.
- 16 Quilici M, Ranque P, Toukara A & Rougemont A. Toxoplasmosis in the republic of Mali. *Acta Trop (Basel)* 33: 239, 1979.
- 17 Sum J C A. *Toxoplasmosis acquisita lymphadenosa*. Munksgaard, Copenhagen, 1961.
- 18 Weinman D & Chandler A H. Toxoplasmosis in man and swine: an investigation of the possible relationship. *JAMA* 161: 729, 1946.
- 19 Work K. Toxoplasmosis. With special reference to transmission and life cycle of *Toxoplasma gondii*. *Acta Pathol Microbiol Scand (B)* 271: 1, 1971.
- 20 Hutchinson N, Dunachie K, Work K and Sum J C. The life cycle of the coccidian parasite *Toxoplasma gondii* in the domestic cat. *Trans R Soc Trop Med Hyg* 65: 380, 1971.

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are common. Among the Skolt Lapps practically each family has at least one cat.

The Lapps also have different eating habits compared with the populations in other parts of Scandinavia. Again this difference is most pronounced among the Skolt Lapps. Fresh meat, milk and eggs are rare and the main foods are fish, bread and flour.

DISCUSSION

Human toxoplasmosis is a fairly common disease in Sundbyberg but rare in Luleå and Umeå in northern Scandinavia. The reason for this is far from clear. The populations studied vary with regard to ethnic origin and environmental factors. It cannot be excluded that both climate conditions and isolation play a role for the low frequency of human infections in the North. An interesting observation is the close contact with cats in the Lappish families. In Stockholm around 50% of the cats are dye test positive (Hult unpublished data). We have so far not been able to get permission from the Lapps to perform a serologic study on their cats. However, Korhonen et al (14) have shown that a wide variety of wild and domesticated animals in Finnish Lapland have antibodies to toxoplasma and it has been demonstrated that 60% of the reindeer in the north of Sweden were DT positive (Hult unpublished). These findings indicate that toxoplasmosis might circulate in nature in the North but that owing to climate conditions or nutritional habits among the Lapps transmission from animal to man only seldom occurs.

We could not demonstrate any correlation between the occurrence of toxoplasmosis and cats in the families in Sundbyberg. Although our findings do not take into account the duration or intensity of exposure to cats it is difficult to see how the failure to estimate these quantitative factors could obscure a real relationship.

Our results suggest a slight familial relationship of the disease. It may be explained by genetic factors and/or a common source of in-

fection within the homes. If however interhuman contact or common source of infection in the homes played a major role in the epidemiology we would expect a higher infection rate in the early childhood. The relatively sharp rise in incidence in adult life suggests that the source of infection lies outside the homes (cf 15, 16, 19).

It has earlier been shown that in Scandinavia maternal females are more often infected than males (8, 9). In our material the sex difference is observed already in small children and becomes marked in puberty and adolescence. This may be due to hormonal differences and/or living habits. However the fact that such sex distribution is not demonstrated in for instance materials from the United States speak against hormonal influence. The theory that women are infected when eating raw meat could not be sustained in our study although this possibility cannot be excluded either. It seems however that infected meat and oocysts from cat are not the only sources of infection.

The disease is thus not uncommon in children and adolescents in Sundbyberg. The infection was most often subclinical like in adults (6, 17). An elevated antibody titer in a child should therefore not be interpreted as a sign of actual disease unless the child has clinical symptoms. A relevant change in antibody titer combined with demonstration of specific IgM and/or IgA is a positive isolation of the parasite.

SUMMARY

1. In Sundbyberg, a suburb of Stockholm, subclinical toxoplasmosis occurs already in early childhood and becomes more frequent in adolescence and especially in adult life.
2. Girls and especially adult women are more often infected than boys and adult men.
3. There is a familial clustering of the disease (albeit of doubtful statistical significance) but the data indicate that infection is most often acquired outside the home.

- 4 No correlation between presence of anti bodies and the exposure to cats or the eating of raw meat could be demonstrated
- 5 Toxoplasmosis is quite rare in Lapps and Skolt Lapps in northern Finland and Norway. The reason for this could not be established

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REFERENCES

- 1 Beverley J K, Fleck D M, Kwantes W & Ludlam G B. Age-sex distribution of various diseases with particular reference to toxoplasmic lymphadenopathy. *J Hyg (Camb)* 76: 715, 1976.
- 2 Christie E, Dubey J P & Pappas P W. Prevalence of Sarcocystis infection and other intestinal parasites in cats from a humane shelter in Ohio. *J Am Vet Med Assoc* 168: 471, 1976.
- 3 Desmonts G, Couvreur J, Ahson F, Baudelot J & Lelong M. Etude épidémiologique sur la toxoplasmose de l'influence de la cuisson de viandes de boucherie sur la fréquence de l'infection humaine. *Rev Fr Etud Clin Biol* 208: 100, 1969.
- 4 Dubey J P, Miller N L & Frenkel J A. The Toxoplasma gondii oocyst from cat feces. *J Exp Med* 133: 636, 1970.
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- 6 Feldman H A. Epidemiological aspects of toxoplasmosis. In *Human Toxoplasmosis* pp. 169-174. Munksgaard, Copenhagen, 1960.
- 7 Hambræus L. Occurrence of antibodies against toxoplasma and tularensis in a country of Northern Sweden. *Communication 2nd Int Symp Circumpol Health Oulu* 1971, p. 48.
- 8 Hedenström G, Hult G & Lagercrantz R. Toxoplasmosis in children. *Acta Paediatr Scand* 30: 304, 1961.
- 9 Haldt G, Johansson M O & Lantto S. Echinococcosis in Northern Scandinavia. Immune reactions in Kautokeino Lapps. *Arch Environ Health* 26: 36, 1973.
- 10 Hutchinson W M. Experimental transmission of toxoplasma gondii. *Nature (Lond)* 206: 961, 1965.
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- 12 Jacobs L, Remington J S & Melton M L. A survey of meat samples from swine, cattle and sheep for the presence of encysted toxoplasma. *J Parasitol* 46: 23, 1960.
- 13 Kean B H, Kimball A C L & Christenson W N. An epidemic of acute toxoplasmosis. *JAMA* 208: 1007, 1969.
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- 17 Sum J C A. Toxoplasmosis acquisita lymphadenosa. Munksgaard, Copenhagen, 1961.
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- 19 Work K. Toxoplasmosis. With special reference to transmission and life cycle of Toxoplasma gondii. *Acta Pathol Microbiol Scand (B)* 271: 1, 1971.
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The Lapps also have different eating habits compared with the populations in other parts of Scandinavia. Again this difference is most pronounced among the Skolt Lapps. Fresh meat, milk and eggs are rare and the main foods are fish, bread and flour.

DISCUSSION

Human toxoplasmosis is a fairly common disease in Sundbyberg but rare in Årviokemo and Inari in northern Scandinavia. The reason for this is far from clear. The populations studied vary with regard to ethnic origin and environmental factors. It cannot be excluded that both climate conditions and isolation play a role for the low frequency of human infections in the North. An interesting observation is the close contact with cats in the Lappish families. In Stockholm around 50% of the cats are dye test positive (Hult unpublished data). We have so far not been able to get permission from the Lapps to perform a serologic study on their cats. However, Korhonen et al (14) have shown that a wide variety of wild and domesticated animals in Finnish Lapland have antibodies to toxoplasma and it has been demonstrated that 60% of the reindeer in the north of Sweden were DT positive (Hult unpublished). These findings indicate that toxoplasmosis might circulate in nature in the North but that owing to climate conditions or nutritional habits among the Lapps transmission from animal to man only seldom occurs.

We could not demonstrate any correlation between the occurrence of toxoplasmosis and cats in the families in Sundbyberg. Although our findings do not take into account the duration or intensity of exposure to cats it is difficult to see how the failure to estimate these quantitative factors could obscure a real relationship.

Our results suggest a slight familial relationship of the disease. It may be explained by genetic factors and/or a common source of infection within the homes.

If however inter human contact or common source of infection in the homes played a major role in the epidemiology we would expect a higher infection rate in the early childhood. The relatively sharp rise in incidence in adult life suggests that the source of infection lies outside the homes (cf 15, 16, 19).

It has earlier been shown that in Scandinavia materials females are more often infected than males (8, 9). In our material the sex difference is observed already in small children and becomes marked in puberty and adolescence. This may be due to hormonal differences and/or living habits. However the fact that such sex distribution is not demonstrated in for instance materials from the United States speak against hormonal influence. The theory that women are infected when eating raw meat could not be sustained in our study although this possibility cannot be excluded either. It seems however that infected meat and oocysts from cat are not the only sources of infection.

The disease is thus not uncommon in children and adolescents in Sundbyberg. The infection was most often subclinical like in adults (6, 17). An elevated antibody titer in a child should therefore not be interpreted as a sign of actual disease unless the child has clinical symptoms. A relevant change in antibody titer combined with demonstration of specific IgM and/or IgA is a positive isolation of the parasite.

SUMMARY

- 1 In Sundbyberg, a suburb of Stockholm, subclinical toxoplasmosis occurs already in early childhood and becomes more frequent in adolescence and especially in adult life.
- 2 Girls and especially adult women are more often infected than boys and adult men.
- 3 There is a familial clustering of the disease (albeit of doubtful statistical significance) but the data indicate that infection is most often acquired outside the home.

A BOY WITH SEVERE INFANTILE GASTROGEN LACTOSE INTOLERANCE AND ACQUIRED LACTASE DEFICIENCY

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ABSTRACT Berg N O Dahlqvist A and Lindberg T (Departments of Pathology and Nutrition University of Lund and Department of Paediatrics Malmö General Hospital Malmö University of Lund Sweden) A boy with severe infantile gastrogen lactose intolerance and acquired lactase deficiency *Acta Paediatr Scand* 68 751 1979 — A 10-year-old boy with severe familial lactose intolerance in infancy (vomiting failure to thrive lactosuria (5.25 g/l) sucrosuria (12 g/l) and aminoaciduria. Intestinal disaccharidases (including lactase and sucrase) normal at age 6 and 20 weeks. Oral lactose tolerance test at this age resulted in lactosuria (4.6 g/l) sucrose tolerance test in sucrosuria (18.5 g/l). In contrast intraduodenal lactose tolerance test gave only low lactose excretion in urine (0.28 g/l). He improved rapidly and had no lactosuria on intraduodenal feeding with citric acid milk. The lactosuria diminished as age increased but was still higher at age 6 years than that of controls. He tolerated normal disaccharide containing food after 1.5 years of age. At 5.5 to 6 years he had symptoms of lactose malabsorption and an isolated lactase deficiency was proved. At 10 years he still tolerates only limited amounts of milk. The defect in severe familial infantile lactose intolerance seems to be localized in the gastric mucosa. Acquired lactase deficiency can appear later in childhood in this syndrome.

KEY WORDS Lactose intolerance lactosuria sucrose intolerance sucrosuria disaccharide intolerance lactase deficiency

Durand in 1958 (13) described a condition he called idiopathic lactosuria. It has since been called severe infantile familial lactose intolerance and is characterized by a critically ill infant with vomiting and failure to thrive disacchariduria and amino aciduria (17, 18). The cause of this rare (about 20 cases have been reported) and probably hereditary disorder is still obscure. Transient lactase deficiency and increased permeability of the mucosa of the small intestine to lactose has been suggested (16, 13, 17, 18). In a short communication in 1969 we reported normal intestinal lactase activity in a boy in the acute phase of this disorder (4). Moreover no lactosuria occurred when the lactose was given intraduodenally suggesting an increased permeability for lactose in the gastric mucosa.

We present here a full account of the studies performed in this boy and a 10-year follow up

METHODS

Lactose tolerance test (LTT) and sucrose tolerance test (STT) were performed after the boy fasted for 6 h (in infancy) or overnight (childhood) with a dose of 2 g/kg body weight in 10% solution. Glucose-galactose tolerance test (GGTT) was performed with a dose of 1+1 g/kg body weight in 10% solution. Intraduodenal LTT. Lactose was given through a feeding tube in pars descendens of duodenum under fluoroscopic control.

Urine was collected the night before the tolerance tests and 0 to 6 h and 6 to 12 h after. The urine was either analysed directly or immediately frozen and stored at -10°C until analysis.

Blood glucose was determined in capillary blood samples taken at 0, 15, 30, 45 and 60 min.

Galactose in urine was determined with galactose dehydrogenase (Boehringer AG Mannheim West Germany) (11) as described by Dahlqvist & Svenningsen (10). Glucose in urine was assayed according to Tengström (33). The urine was first filtered through a column of ion exchange resin and then analysed with a glucose oxidase reagent. Lactose in urine was determined from the increase in galactose after incubation with a fungal lactase (β -galactosidase) (11). 5 ml of urine was mixed with 0.5 ml of lactase solution (Maxilact[®] Gist Brocades Delft). The

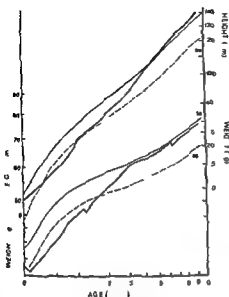


Fig. 2 Weight and height curves of the patient. Mean (\pm) and \pm 3 S.D. curves of normal children (23) are given for comparison.

S-P and S-ALP normal. S-urea normal. urinary osmolality varied from low to normal.

At 17 months he tolerated lactose containing foods. At 18 months 300 ml milk per day. After age 4 years he had a normal diet which included up to 1 litre milk/day. His

weight and height increased and the 50th percentile for weight was reached at 3 years and for height at 4 years (Fig. 2).

At 5.5 years he got loose and foul smelling stools. Weight standstill. Cluotest of faeces was 0.5%. Xylose tolerance test was normal (2 mmol/l at 30 min and 2.9 mmol/l at 60 min) as was lactose tolerance test (blood glucose increase of 2 mmol/l within 60 min). B-folic acid normal. Faecal chymotrypsin normal. No enteropathogenic microorganisms in faeces. Periodically diarrhoea continued. Small intestinal biopsies at 6 and 6.5 years show decreased lactase activity (see below). With a lower lactose intake (about 200 ml milk/day) he became free of symptoms. These returned when he drank 4–500 ml milk/day. His weight and height were normal (Fig. 2) his condition good.

SPECIAL INVESTIGATIONS

Urinary excretion of carbohydrates

Table 1 presents the excretion of glucose, galactose, lactose and sucrose in urine when the patient was on various diets. The values from the controls are given as a comparison. Large amounts of lactose and sucrose were excreted during the first month when a lactose or sucrose containing diet was given. The disacchariduria disappeared on a lactose and sucrose free diet. The lactosuria reappeared

Table 1 Urinary excretion of carbohydrates (g/l) in the patient, the parents, the sister and controls

	Age	Diet	Glucose	Galactose	Lactose	Sucrose
Patient	1 m	Human milk		0.040	5.750	
	2 m	Nutramigen			0.080	8.100
	3 m	Nutramigen	0.510			11.940
	3½ m	DSI	0.078	0.010	0.017	0
	6 m	Citrado intraduod.		0.036	0.080	0.670 ^a
	7 m	Citrado per oral		0.04	0.178	
	8 m	Citrado per oral	0.174	0.054	0.340	0.064
	9 m	Findus valling	0.096	0.030	0.164	0.074
	10 m	Glucose formula	0.386	0.00	0.040	0.012
	1½ y	Glucose formula		0.010	0.036	
	4 y	Normal	0.080	0.078	0.174	
	4½ y	Normal	0.046	0.07	0.137	
	6 y	Normal		0.00	0.250	
Mother	0 y	Normal		0.040	0.048	
Father	3 y	Normal		0.040	0.048	
Sister	5 d	Human milk		0.157	1.110	
Controls n=9	1–3 m	Human milk or cow's milk based formula		0.041	0.711	
	mean value (range)			(0.016–0.078)	(0.016–0.47)	

^aNutramigen®=sucrose containing formula (Mead Johnson). DSI=monosaccharide (glucose) formula (Nestlé). Citrado=citric acid milk. Findus valling=cow's milk based formula (AB Findus). Glucose formula=besides the sugar similar to fructose formula (L.S.).

After 3 ml of a sucrose-containing syrup.

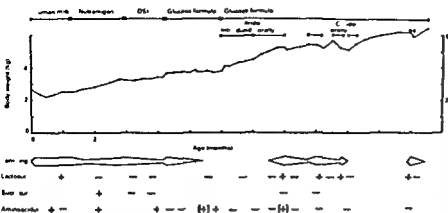


Fig. 1 Survey of the clinical course during the first year of life. Nutramigen® = sucrose containing formula (Mead Johnson) DSI = monosaccharide (glucose) formula (Nestlé) kindly supplied by Professor B. Lindqvist. Citricid = citric acid milk. F V = Findus Valling cow's milk based formula (AB Findus). Glucose formula = apart from the sugar similar to fructose formula (25).

Netherlands) 4 mg/ml in 0.1 mol/l K-phosphate buffer pH 6.8). After incubation at 30°C for 1 h the galactose concentration was assayed as described above. Sucrose in urine was determined from the increase in glucose after incubation with yeast invertase (sucrase β -fructosidase) 0.5 ml of urine was mixed with 0.5 ml of invertase solution (yeast invertase 10 mg/ml in 0.2 mol/l Na acetate buffer pH 4.5 Nutritional Biochem. Corp.) After incubation for 30 min the glucose concentration was assayed as described above.

Paper chromatography of urine. The sugars in urine were also identified with semiquantitative paper chromatography. As solvent we used ethyl acetate:acetic acid:water 9:2:2 (deionization of the urine is not needed with this reagent). The spots were developed with aniline phthalate (28). α -Amino nitrogen in urine was measured as described by Kachadurian et al. (22).

Amino acids in urine were determined with high voltage electrophoresis on paper (16, 30).

Other laboratory tests included daily faecal fat determination, Clinitest and pH of faeces, D-xylose tolerance test, trypsin in duodenal juice, faecal chymotrypsin, sweat test, S-Ca, Σ P, Σ ALP, S-ALAT, S-ASAT, Σ urea and culture of faeces for enteropathogenic microorganisms (for ref. see 3). Small intestinal biopsies were taken by a hydraulic capsule at the duodenal flexure under fluoroscopic control (3). Specimens for enzyme analyses were kept at -20°C. Specimens for light microscopy were handled as described earlier (3). Gastric mucosal biopsies were taken by the same capsule under fluoroscopic control for light and for electron microscopy.

Intestinal disaccharidases were determined according to Dahlqvist (7) and intestinal dipeptidases according to Josefsson & Lindberg (21) and Lindberg et al. (24).

Controls consisted of 9 infants, 3 weeks to 3 months of age and with the following diagnosis: uncomplicated prematurity (1), pyloric stenosis (3), chelio gnathopalatoschisis (1), healthy infants admitted for social reasons or illness of the mother (3).

CASE REPORT

Boy born at term, birth weight 2650 g, first child of healthy Swedish parents with a healthy younger sister. He was fed human milk but vomited and lost weight. He

was referred to the Department of Paediatrics because of these symptoms at 10 days of age. During the following weeks his main symptoms were excessive vomiting and failure to thrive. His stools were normal, pH of faeces 6-7. Clinitest of faeces negative. X-ray of the oesophagus and the stomach at age 2 weeks revealed a slight elongating and narrowing of the pyloric canal. At age 5 weeks a second X-ray of the stomach and small intestine was normal.

Clinitest of urine was positive, the sugar was lactose (see below). He had amino aciduria (see below). He began to gain in weight, especially when he had a lactose free formula Nutramigen® (Mead Johnson) instead of human milk at 6 weeks of age. Fig. 1 surveys the clinical course and the various dietary trials during the first year. At 5 months of age vomiting stopped when he had a glucose formula (besides the sugar similar to fructose formula (25)) and a saccharose and lactose free diet. He got otitis and urinary tract infections (X-ray of the urinary tract normal). Weight standstill. As he had no symptoms and no lactosuria when the lactose was given intraduodenally (see below) citric acid milk (Citrid) was given intraduodenally (fluoroscopic and X-ray control) with a feeding tube from 3 to 7 months of age. This resulted in weight gain and he improved clinically. He did not vomit and had no lactosuria. At 7 months he tolerated increasing amounts of citric acid milk orally. At 8 months he had a relapse, began to vomit and got lactosuria. Lactose and saccharose free diet was given but during the following month he deteriorated, got fever and urinary tract infection and excessive vomiting. He was dehydrated. Fluid was given intravenously for 2 days. Then citric acid milk was given intraduodenally for some days resulting in a rapid weight gain. Successively clinical improvement at one year of age, his weight was 6.2 kg, skeletal age delayed, he could hardly sit without support, his mental development was normal. He had no hepato-splenomegaly. Ophthalmological examination normal. Results of various laboratory tests performed during this first year were: Faecal fat 2 g/d, xylose tolerance test normal (1.9 mmol/l at 30 min and 2.3 mmol/l at 60 min). Lactose tolerance tests normal, increase of B-glucose (see below) trypsin in duodenal juice normal $\times 2$ (300 and 540 μ g/ml), faecal chymotrypsin normal, sweat test normal, S-ASAT and S-ALAT normal except for a transitory increase at 5 to 7 months of age, Σ bilirubin normal, S-Ca

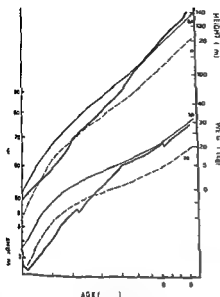


Fig 2 Weight and height curves of the patient. Mean (\bar{x}) and -3 S.D. curves of normal children ($n=73$) are given for comparison.

S-P and S-ALP normal. S-urea normal. Urinary osmolality varied from low to normal.

At 17 months he tolerated lactose-containing foods. At 18 months 300 ml milk per day. After age 7 years he had a normal diet which included up to 1 litre milk/day. His

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Table 1 presents the excretion of glucose, galactose, lactose and sucrose in urine when the patient was on various diets. The values from the controls are given as a comparison. Large amounts of lactose and sucrose were excreted during the first month when a lactose or sucrose containing diet was given. The disacchariduria disappeared on a lactose and sucrose free diet. The lactosuria reappeared

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	2½ m	Nutramigen	0.510			11.940
	3½ m	DSI	0.08	0.010	0.01*	0
	6 m	Citrido intraduod		0.036	0.080	0.670*
	7 m	Citrido per oral		0.074	0.178	
	8 m	Citrido per oral	0.124	0.054	0.540	0.064
	9 m	Findus valling	0.096	0.030	0.164	0.074
	10 m	Glucose formula	0.386	0.070	0.040	0.012
	1 y	Glucose formula		0.010	0.036	
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	4 y	Normal	0.046	0.02	0.137	
	6 y	Normal		0.00	0.50	
Mother	0 y	Normal		0.040	0.048	
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Sister	5 d	Human milk		0.157	1.110	
Controls	1–3 m	Human milk or cow's milk based formula		0.041	0.211	
mean value (range)				(0.016–0.078)	(0.016–0.47)	

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After 5 ml of a sucrose-containing syrup

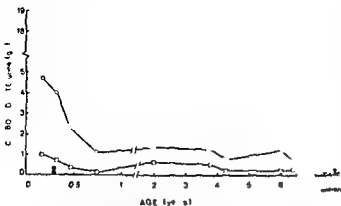


Fig. 3 Carbohydrate excretion in urine at lactose and sucrose tolerance tests at various ages of the patient and in the controls (age 1–3 months $n=9$). O=lactose □=galactose △=sucrose ●=lactose intraduodenal LTT ■=galactose intraduodenal LTT

when citric acid milk was given orally at 7 to 8 months of age. However the same amounts of citric acid milk given intraduodenally resulted in a considerably lower lactose excretion in urine.

The parents had no increase in galactose and lactose in urine. His sister at 5 days of age had 1.1 g/l lactose in urine, which is within normal limits for this age (5 Dahlqvist & Svenningsen unpublished). Later the Clini test was negative. She had no symptoms.

Carbohydrate tolerance tests

All carbohydrate tolerance tests gave an increase between 1.2 and 4.95 mmol/l of blood glucose within 60 min (normal >1.1 mmol/l). Urinary excretion of galactose at glucose–galactose tolerance test (GGTT) at 2 months was 1.55 g/l and of glucose 0.4 g/l. Fig. 3 shows the urinary excretion of lactose and galactose at lactose tolerance tests (LTT) at various ages of the patient and of 8 control infants. It is evident that the patient's lactose excretion is high and considerably greater especially at age 2 to 5 months than that of the controls (3 weeks to 3 months of age). In contrast the lactose excretion was low at the intraduodenal LTT at 3.5 months. The lactose excretion at intraduodenal LTT was higher in the two controls (3 and 4 weeks of age and prematurely born). The result of their per oral

LTT did not differ from those of the other controls. The urinary glucose concentration in the various LTT varied between 0.02 to 0.41 g/l. At the GGTT and LTT at 2 months of age the boy had a sucrose containing diet (Nutramigen®). The urinary sucrose excretion at these two tests increased from 3.7 to 14.2 g/l and from 6.0 to 13.8 g/l respectively. Fig. 3 also shows the patient's extremely high sucrose excretion at the sucrose tolerance test (STT) at 2.5 months. This contrasts to the low urinary sucrose concentration at STT in the control infant of the same age.

There was no immediate clinical reaction at the oral tolerance tests but in infancy the vomiting frequency temporarily increased. When older he had no symptoms. Intraduodenal LTT gave no reaction.

LTT in the parents gave a normal B glucose rise and no abdominal symptoms. The father's lactose in urine increased from 0.048 to 0.1 g/l, the mother's from 0.048 to 0.096 g/l. Corresponding figures for galactose were 0.04 to 0.174 and 0.04 to 0.18 g/l respectively.

Amino acid excretion in urine

During the first months he had cystathioninuria. All tolerance tests at this age resulted in an increased excretion of cystathionine. When he got sucrose containing formula (Nutramigen®) and at the STT he had a generalized amino aciduria (α amino nitrogen 700 mg/g creatinine). At the STT the α amino nitrogen increased from 397 to 600 mg/g creatinine. After 8 months there were no abnormalities in urinary amino acid excretion. The parents had normal amino acid excretion in urine.

Small intestinal biopsy

Small intestinal biopsies were taken at 6 and 20 weeks and at 6 and 6.5 years of age. The biopsy from 6 weeks showed ridges and leaves and also a small flat area in dissecting microscopy. The flat area was almost avillous and showed a heavy plasma cell infiltration (Fig. 4a). Other areas in the same specimen had a villous mucosa with slightly reduced height.



Fig. 4 (a) Small intestinal biopsy at 6 weeks of age. A flat area with elongation of the crypts and infiltration with plasma cells. (b) Small intestinal biopsy at 6 weeks of age. Another part of the biopsy specimen shown in Fig. 4a.

Villous structure, slight irregularities in surface epithelium. (c) Small intestinal biopsy at 6 years of age. Normal intestinal mucosa.

and nuclear irregularities of villous epithelium (Fig. 4b). The other biopsies showed a normal mucosa (Fig. 4c). Table 2 shows the intestinal disaccharidase activities in the various biop-

sies. The lactase activity was normal in biopsies from 6 and 20 weeks of age, but low in biopsies from 6 and 5 years. The other enzyme activities were within the ranges of the

Table 2 *Intestinal disaccharidase activities in small intestinal biopsies from the patient at various ages*

	6 w	20 w	6 y	6.5 y
Maltase μg protein (91-600)	116	51	120	102
Isomaltase μg protein (20-126)	46	28	43	33
Sucrase μg protein (23-166)	48	29	27.3	25.4
Trehalase μg protein (8.3-50)	4.4	11.1	8.6	6.2
Lactase μg protein (6.6-73)	15.8	24.8	2.9	3.7

* Controls range ($n=49$) (8)

controls. The intestinal dipeptidase activities on glycyl L leucine, L-alanyl L glutamic acid, L-alanyl L glutamic acid and L-alanyl L proline were analysed in the biopsy from 6 weeks of age and were within normal limits.

Gastric biopsy

Light microscopy and electron microscopy of biopsies from fundus and corpus of the stomach taken at 20 and 21 weeks of age showed no abnormalities.

DISCUSSION

The main features in severe familial lactose intolerance are the excessive vomiting and the lactosuria during the first months of life. These were also our patient's dominant symptoms. Moreover, he had a pronounced sucrosuria on a diet rich in sucrose. This agrees with findings in several other patients (2, 6, 11, 20, 29). The lactose and sucrose concentration in urine was occasionally up to 100 times greater than normally found at this age (5). Lactose intolerance is reported to disappear at 6 to 18 months after onset (17). This agrees with our experience. The lactosuria in the boy decreased with increasing age as was clearly demonstrated by the lactose excretion in urine at LTT at various ages (see Fig. 3). However, the lactose concentration in urine at LTT was always higher than those of the controls and also

higher than reported in the literature (15). On normal diet from 2 to 6 years of age the lactose concentration in urine was slightly higher than normally found (5).

This pronounced disacchariduria in infancy could be caused by the disaccharides not being hydrolysed because of disaccharidase deficiency but absorbed as such across the small intestinal mucosa. However, the patient's lactase and sucrase activities were normal at 6 and 20 weeks of age and also in other patients studied in the acute phase of the disease (1, 2, 14). Moreover, LTT in several patients including the present one (1, 2, 6, 11, 19, 29) resulted in a normal increase in blood glucose. These findings exclude the possibility that the disease is caused by a transient disaccharidase deficiency.

The intraduodenal LTT showed that the lactose was split by the lactase in the intestinal mucosa. This test gave a normal blood glucose increase and of more interest was not followed by an abnormal lactosuria. The urinary lactose concentration was about one twentieth of the concentration when the lactose was given orally and was comparable to that at per oral LTT of the controls. (The higher urinary lactose excretion at the intraduodenal LTT in the two controls can be explained by their prematurity and younger age, i.e. the lactase activity not being fully developed.) In accordance with the result of the intraduodenal LTT the boy was symptom-free, improved clinically and had no abnormal lactosuria when cow's milk (citric acid milk) was given intraduodenally between 6 and 7 months of age. The symptoms and lactosuria returned when he then had cow's milk orally. Russo et al. (29) have confirmed these findings. At 8 months of age two intraduodenal LTT gave no lactosuria in their patient whereas two oral LTT were followed by lactosuria.

Thus it seems that the defect in the gastrointestinal tract is localized more orally than in the duodenum and then most probably in the stomach. Light and electron microscopy of

gastric mucosa from the fundus-corpus region was normal. The morphology of the antral mucosa was not studied. Further studies of the gastric mucosa are needed before reaching any conclusions on the morphology of the gastric mucosa. In this context the observations of Moncrieff & Wilkinson (26) and of Woodruff (32) are of interest. They found sucrosuria in mentally retarded children who vomited and had hiatus hernia. The children also had lactosuria and one patient vomited excessively and collapsed on lactose (26). Moncrieff & Wilkinson (26) showed that when lactose was given together with sucrose in the diet the sucrosuria became more pronounced. This agrees with our findings.

The coexistence of pyloric stenosis reported in some infants with severe familial lactose intolerance (11, 20) is difficult to explain. The first X-ray of our patient's stomach showed slight signs of pyloric stenosis; at the second X-ray some weeks later these had disappeared. Another infant (6) also had slight signs of pyloric stenosis but this could not be verified. The occurrence of lactosuria in infants with pyloric stenosis has long been known (27). Out of 150 infants with pyloric stenosis Darling et al. (11) found reducing substances in urine in one. Bichel (5) reported pyloric stenosis among the disorders with excretion of lactose, galactose, glucose and fructose. It can be speculated upon whether the combination of a defect in the gastric mucosa as in severe familial lactose intolerance or an irritation of the gastric mucosa as in hiatus hernia with an abnormal motility of the stomach can lead to the abnormal absorption of the disaccharides in the stomach.

The amino aciduria, galactosuria and other signs of liver and renal damage are probably secondary phenomena caused by the disaccharides (5, 6, 11).

Interestingly the boy at 5.5 years of age had clinical symptoms of lactose malabsorption. Isolated decreased lactase activity was found in two biopsies taken with a 6 month interval and he has still at 10 years of age a limited

tolerance for lactose. The cause of this acquired lactase deficiency is obscure. He had no signs of gastroenteritis, of exocrine pancreatic insufficiency or of malnutrition. His parents are of Swedish extraction and acquired lactase deficiency at this age in Swedish children is rare (9). Thus a coincidence is not a plausible explanation. It has not been reported before in severe familial lactose intolerance but there are no other reports of such a long follow-up. Holzel (18) reported normal lactase activity after recovery of the acute phase of the disease but his patient was younger at biopsy. Russo et al. (29) reported from Sicily that four adult male family members had cow's milk intolerance and that 250 ml of cow's milk orally gave each member abdominal pain and lactosuria. Two of eight controls got abdominal pain but no lactosuria. Their patient and affected family members and also bilateral cataracts not reported earlier in this disease.

In conclusion the results from our study indicate that the basic defect in severe familial lactose intolerance might be localized to the gastric mucosa. We suggest that the treatment in the acute phase is a formula free from disaccharides or intraduodenal feeding with a disaccharide containing formula. Lactose malabsorption due to lactase deficiency can develop later in childhood.

REFERENCES

1. Arakawa T, Akabane J & Akabane M. Lactose intolerance with normal activity of lactase. *Tohoku J Exp Med* 111: 165, 1963.
2. Arafshana M, Matsuda I, Sato N & Yoshioka H. Intestinal disaccharidase activity in an infant with lactose intolerance. *Helv Paediatr Acta* 26: 115, 1971.
3. Berg N O, Borulf S, Jakobsson I & Lindberg T. How to approach the child suspected of malabsorption. *Acta Paediatr Scand* 67: 403, 1978.
4. Berg N O, Dahlqvist A, Lindberg T & v. Stodtitz W. Severe familial lactose intolerance—a gastrointestinal disorder? *Acta Paediatr Scand* 58: 575, 1969.
5. Bichel H. Mellituria: a paper chromatographic study. *J Pediatr* 59: 641, 1961.
6. Carlson N A J & Neely W A. Disaccharide intolerance in infancy. *Arch Dis Child* 38: 574, 1963.
7. Dahlqvist A. Assay of intestinal disaccharidases. *Anal Biochem* 72: 99, 1968.

- Dählqvist A, Lindberg T, Meeuwisse G & Åkerman M. Intestinal dipeptidases and disaccharidases in children with malabsorption. *Acta Paediatr Scand* 59 621 1970
- 9 Dählqvist A & Lindqvist B. Lactose intolerance and protein malnutrition. *Acta Paediatr Scand* 60 488 1971
- 10 Dählqvist A & Svenningsen N W. Galactose in the urine of newborn infants. *J Pediatr* 75 454 1969
- 11 Darling ■, Mortensen O & Sondergaard G. Lactosuria and amino aciduria in infancy. *Acta Paediatr Scand* 49 281 1960
- 12 Duodoroff M. D-Galactose dehydrogenase of *Pseudomonas saccharophila*. In ■ P Colowick & N O Kaplan (eds.) *Methods in Enzymol* vol 5 Academic Press, New York 1962 p 339
- 13 Durand P. Lattosuria idiopathica in una paziente con diarrea cronica ed acidosi. *Minerva Pediatr* 10 706 1958
- 14 Eggermont E & Bracke P. Etude clinique et enzymatique de la maladie de Durand. XXII Congr de l'Association des Pédiatres de langue Française Strasbourg 1969
- 15 Haworth J C. Sugars in the blood and urine of children following the ingestion of disaccharides. *Arch Dis Child* 35 552 1960
- 16 Holmgren G, Jeppson J O & Samuelsson G. High voltage electrophoresis in urinary amino acid screening. *Scand J Clin Lab Invest* 26 313 1970
- 17 Holzel A. Sugar malabsorption due to deficiencies of disaccharidase activities and of monosaccharide transport. *Arch Dis Child* 42 341 1967
- 18 Holzel A. Disaccharide intolerances. In ■ S Holt & V P Coffey (eds.) *Some Recent Advances in Inborn Errors of Metabolism* Livingstone, Edinburgh 1968 p 101
- 19 Holzel A, Mereu T & Thomson M L. Severe lactose intolerance in infancy. *Lancet* ii 1346 1962
- 20 Inall M B & Burkinshaw J H. Lactosuria and sucrosuria with failure to thrive. *Proc R Soc Med* 53 318 1960
- 21 Josefsson L & Lindberg T. Intestinal dipeptidases I. Spectrophotometric determinations and characterization of dipeptidase activity in pig intestinal mucosa. *Biochim Biophys Acta* 105 149 1965
- 22 Kachadurian A, Knox W E & Cullen A M. Colorimetric ninhydrin method for total alpha amino acids of urine. *J Lab Clin Med* 56 371 1960
- 23 Karlberg P, Taranger J, Engstrom I, Lichtenstein H & Svennberg Redegren I. The somatic development of children in a Swedish urban community. *Acta Paediatr Scand* Suppl 158 77 1976
- 24 Lindberg T, Norden Å & Josefsson L. Intestinal dipeptidases. Dipeptidase activities in small intestinal biopsy specimens from a clinical material. *Scand J Gastroenterol* 3 177 1968
- 25 Lindqvist B & Meeuwisse G. Diets in disaccharidase deficiency and defective monosaccharide absorption. *J Am Diet Assoc* 48 307 1966
- 26 Moncrieff A & Wilkinson R H. Sucrosuria with mental defect and hiatus hernia. *Acta Paediatr Scand* Suppl 100 495 1954
- 27 Rietschel H. Inanition und Zuckerausscheidung im Säuglingsalter. *Z Kinderheilkd* 7 282 1913
- 28 Robinson W M C & Rathbun J C. Electrophoresis of free sugar in blood. *Science* 127 1501 1958
- 29 Russo G, Mollica F, Mazzone D & Santonocito H. Congenital lactose intolerance of gastroenteric origin associated with cataracts. *Acta Paediatr Scand* 63 457 1974
- 30 Studnitz v W. Secondary cystathioninuria. *Acta Paediatr Scand* 58 173 1969
- 31 Tengstrom B. Enzymatic determination of glucose and galactose in urine. *Scand J Clin Lab Invest* 18 Suppl 97 104 1966
- 32 Woodruff G G. Sucrosuria in association with mental deficiency and hiatal hernia. *J Pediatr* 57 66 1958

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SHORT COMMUNICATION

LEAD INTOXICATION AS AN ETIOLOGIC FACTOR IN HYPERKINETIC BEHAVIOR IN CHILDREN A NEGATIVE REPORT

It has been reported that more than half of a group of hyperkinetic children in New York had a high blood lead level (3). Treatment with lead chelating agents improved behavior in these children (4). Several studies have shown that exposure to lead may cause dysfunction in the central nervous system both in man (5, 12, 13, 14, 15) and in animals (11). It has also been found that lead intoxication in children is not confined to urban slums but also occurs in smaller communities (7).

If lead intoxication is an important cause of hyperkinetic behavior in children this will have great consequences for therapy as well as for prophylaxis. In Scandinavian countries no such investigation has been reported previously. In this investigation lead concentration in hair was used as a measure of lead intake since this seems to be a better measure of long term exposure than blood lead level (2, 6).

The patients were 19 boys (age 11.9 ± 2.2 years, mean \pm S.D.) who had been diagnosed as having a hyperkinetic syndrome. Criteria for the diagnosis were the following: 1) Motor hyperactivity, 2) poor impulse control, 3) low frustration tolerance, 4) short attention span, 5) distractibility, 6) aggressiveness, 7) diminished sensitivity to reinforcement, and 8) reduced skin conductance level. Details of the diagnostic criteria have been published previously (10).

Twenty-two boys (age 11.4 ± 0.7 years) were used as controls. Both the patients and the controls lived in the Bergen area (a city of about 210,000 inhabitants); however, the control group was from a geographically more remote area.

Blood lead levels were low in men working in downtown Bergen (1) but high lead concentrations have occasionally been observed in blood, hair and teeth in children (8, N. P. Berg Justesen, data to be published).

Samples of 100 mg of hair were obtained from each subject; these were taken from the same part of the head and at the same distance from the base of the hair. The lead concentration was determined by atomic absorption spectrophotometry as described by others (8, 9).

The hair lead concentration in the hyperkinetic group was $7.4 \pm 5.4 \mu\text{g/g}$ (mean \pm S.D.) and in the control group $8.2 \pm 7.0 \mu\text{g/g}$ ($p > 0.10$, *t*-test). All values observed were well within what is considered to be a normal range (N. P. Berg Justesen, data to be published). The three highest values were in the control group. It has been shown that skin conductance level is reduced in children with a hyperkinetic syndrome (10). Correlations between lead concentrations, basal skin conductance levels and spontaneous activity (10) were therefore calculated (Pearson product moment correlation). No significant correlations were found either in the hyperkinetic group or in the control group.

This investigation indicates that lead intoxication is not an important etiologic factor in hyperkinetic behavior in children in Bergen. This of course does not exclude the possibility that lead intoxication may be of importance in areas with more heavy exposure to lead.

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REFERENCES

- 1 Bjørdal M Romslo I Brinkmann J A & Vollset P Bly og karbonmonoksyd i blod hos anleggsarbeidere i Bergen *Tidsskr Nor Lægeforen* 99 886 1978
- 2 Cresson J P Hinners T A Baumgartner J F & Pinkerton C Trace elements in hair as related to exposure in metropolitan New York *Clin Chem* 21 603 1975
- 3 David O Clark J & Voeller K Lead and hyperactivity *Lancet* II 900 1972
- 4 David O J Hoffman S P Sverd J Clark J & Voeller K Lead and hyperactivity Behavioral response to chelation A pilot study *Am J Psychiatry* 133 1155 1976
- 5 de la Burdè H & Choate M S Early asymptomatic lead exposure and development at school age *J Pediatr* 87 638 1975
- 6 Eatough D J Christensen J J Izatt R M & Hartley C Level of selected trace elements in human hair In A C Brown (ed) *The first human hair symposium* Williams & Wilkins Baltimore 1974 p 377
- 7 Fine P R Thomas C W Suhs R H Cohnberg R E & Flashner B A Pediatric blood lead levels *JAMA* 271 1475 1972
- 8 Fosse G & Justesen N P Berg Lead in deciduous teeth of Norwegian children *Arch Environ Health* 33 166 1978
- 9 Harrison W W Yurachek J P & Benson C A The determination of trace elements in human hair by atomic absorption spectroscopy *Clin Chim Acta* 73 83 1969
- 10 Kløve H & Hole K The hyperkinetic syndrome Criteria for diagnosis In R L Trites (ed) *Hyperactivity in children etiology measurements and treatment implications* University Park Press Baltimore 1979 in press
- 11 Kostas J McFarland D J & Drew W G Lead induced hyperactivity Chronic exposure during the neonatal period in the rat *Pharmacology* 14 435 1976
- 12 Landrigan P J Whitworth R H Baloh R W Staehling N W Barthel W F & Rosenblum B F Neuropsychological dysfunction in children with chronic low level lead absorption *Lancet* I 108 1975
- 13 Pihl R O & Parkes M Hair element content in learning disabled children *Science* 198 704 1977
- 14 Valciukas J A Lilis R Fischbein A Selikoff I J Eisinger J & Blumberg W E Central nervous system dysfunction due to lead exposure *Science* 201 465 1978
- 15 Waldron H A Lead and human behaviour *J Ment Defic Res* 22 69 1978

SHORT COMMUNICATION

CONGENITAL TOXOPLASMOSIS LATE APPEARANCE OF RETINAL LESIONS AFTER TREATMENT

Chorodoretinitis is frequently observed in congenital toxoplasmosis. It is often present at birth but sometimes only develops after several years. As far as we know the late appearance of chorodoretinitis following treatment of subclinical congenital toxoplasmosis has not been reported.

Sophie G. a 7 year old girl is the second child of healthy parents. During the first hours of life she developed purpura, cyanosis, hepatosplenomegaly and hypotonia. *Listeria cytomegalovirus*, rubella and syphilis were excluded. Sabin-Feldman and complement fixation tests were strongly positive. *Toxoplasma* was isolated from the patient's CSF after incubation into mouse peritoneum (1). The diagnosis of congenital toxoplasmosis was confirmed by the IgM IFA test (7). Specific treatment consisted of 21 day courses of pyrimethamine (1 mg/kg) and sulfadiazine (100 mg/kg) alternating with spiramycin (50 mg/kg) until the age of 11 months. Between birth and 2 1/2 years the optic fundi were repeatedly described as normal by experienced ophthalmologists. Neurological and psychomotor development were excellent. At the age of seven years bilateral chorodoretinitis consistent with the diagnosis of toxoplasmosis was discovered during a routine examination. Both fundi showed numerous focal heavily pigmented and sharply demarcated scars. In the right eye there were juxta papillary, juxta foveal and peripheral lesions. In the left eye the lesions were located in the periphery and there was vitreous fibrosis anterior to some of them. Physical examination and laboratory data disclosed no other causal agent. Neither the toxoplasma serology nor the IgM IFA test supported reinfection by toxoplasmosis. The

CSF antibody titre (6) appeared equal to that of serum ruling out local CSF production of antibodies due to reactivation of cerebral toxoplasmosis. Unfortunately the aqueous humor antibody titre (2) could not be determined. A significant rise of ocular antibodies would have been additional proof that the chorodoretinitis was due to toxoplasmosis. In absence of any signs of reinfection by toxoplasmosis no treatment was instituted.

The late appearance of ocular manifestations in congenital toxoplasmosis is well documented (4). As congenital toxoplasmosis is often atypical at birth and because of the late appearance of retinal lesions Desmonts & Couvreur (4) recommend treatment in all newborns suspected of having congenital toxoplasmosis. The literature does not mention the appearance of lesions after correct neonatal treatment. With regard to the late appearance of retinitis in untreated cases it has been suggested (3) that the lesions are due to a delayed hypersensitivity reaction to the encysted parasites resulting in local proliferation and scar formation. Encystation occurs very early after toxoplasmic contamination and while sulfadiazine and pyrimethamine eliminate the trophozoites they do not act on encysted organisms. The efficiency of spiramycin is doubtful and the practical benefit of treatment is still controversial. In a 15 year old girl described by Frezotti et al (5) sulfonamides did not prevent worsening of the existing chorodoretinitis or extension to the other eye. In contrast in eight children with subclinical toxoplasmosis at birth described by Saxon et al (8) only the children who were treated did not develop evidence of intellectual impairment. Finally Desmonts (3) has confirmed our ob-

servation and stated that treatment only reduces the frequency of retinopathy but cannot completely prevent it

We are indebted to Dr Desmonts (Lab. de sérologie neonatale et de recherche sur la toxoplasma à Paris) for his helpful advice

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REFERENCES

- 1 De Meuter F. La toxoplasmose en Belgique. *Mém Acad R Méd Belg*, 2ème série 5: 163, 1967

- 2 Desmonts G. Toxoplasmose oculaire. Etude épidémiologique. *Arch Ophthalmol* (Paris) 33: 87, 1973
- 3 Desmonts G. Personal communication
- 4 Desmonts G & Couvreur J. Congenital toxoplasmosis. A prospective study of 378 pregnancies. *N Engl J Med* 290: 1110, 1974
- 5 Frezotti E, Guerra R, Terragna A & Tosi P. A case of congenital toxoplasmosis with active chorioretinitis. *Ophthalmologica* 169: 371, 1974
- 6 Prussan C, Desmonts G & Mozzioconacci M. Évolutive neurologique tardive d'une toxoplasmose congénitale démontrée par l'étude du LCR. *Ann Pédiatr* 18: 224, 1971
- 7 Remington J S, Miller M J & Brownlee I. M. antibodies in acute toxoplasmosis. II. Prevalence and significance in acquired cases. *J Lab Clin Med* 71: 855, 1968
- 8 Saxon S A, Knight W, Reynolds D W, Stagno S & Alford C A. Intellectual deficits in children born with subclinical congenital toxoplasmosis. *J Pediatr* 92: 792, 1973

SHORT COMMUNICATION

REGRESSION OF WILMS TUMOR AFTER PREOPERATIVE CHEMOTHERAPY

In all cases of Wilms tumor two-year survival rates of more than 80% can now be expected after the combined use of surgery radiation and chemotherapy (1 3 4 5 6 8). However the best way of using and combining these three treatments is still a matter of controversy. In studies initiated by and under the surveillance of the International Society of Pediatric Oncology (SIOP) studies concerning the effect of preoperative chemotherapy were started in the beginning of the 70's. Preliminary results were published in 1976 (8). It was concluded that pre-operative radiation therapy prevented rupture of the tumor at operation which seemed to be correlated to a higher recurrence free survival. In a later study to be published by SIOP preoperative radiation was combined with preoperative chemotherapy. Nephrectomy was followed by multiple courses of combined actinomycin D and vincristine the latter therapeutic regime originally suggested by D'Angio et al (2). The results in this SIOP study appear to be superior to that in the earlier SIOP study. However preoperative radiation may have late side effects. Therefore it might be preferable to omit preoperative radiotherapy in favour of preoperative chemotherapy alone. Sullivan et al (9) had already by 1967 replaced preoperative radiation with chemotherapy alone and had shown marked effects on the tumors. Therefore in 1977 SIOP initiated a prospective study to investigate whether pre-operative chemotherapy alone without radiation can maintain the good results regarding tumor rupture at operation and the overall long term survival. The outcome of this study in which we are taking part will of course not be available in

several years. We have however observed a remarkable regression of Wilms tumor after preoperative chemotherapy alone in our first four consecutive patients and therefore a preliminary report seems indicated.

The patients were 6 months 2 2 5 and 7 5 years respectively. The preoperative treatment consisted of intravenously administered actinomycin D and vincristine over a period of three weeks. Actinomycin D was given in a dose of 15 µg/kg body weight on day 1 2 3 14 16 and 17 and vincristine in a dose of 1 5 mg/kg body weight on day 1 8 15 and 22. Angiography was performed both before treatment started and on the day of operation which was scheduled one week after the chemotherapy was completed. Selective angiography was performed via catheterization of the renal artery as well as other aortic branches that might supply the tumor. At initial angiography vascular supply from the renal artery was graded as gross in three cases and moderate in one. Two tumors also had a moderate vascular supply from lumbar arteries. Angiography after chemotherapy demonstrated a remarkable decrease in tumor vascularity in all children and in two tumor vessels were completely absent. There was a marked reduction in the bulk of the tumor in all cases. In 3 out of the 4 children angiography after chemotherapy demonstrated considerably more intact renal parenchyma than before treatment. At nephrectomy slight perirenal oedema was found but no fibrosis. Three of the kidneys were easily removed but one largely necrotic tumor ruptured. According to the SIOP staging classification of Wilms tumor two of the tumors were stage I (one

highly and one moderately differentiated) one stage II and one stage III (both moderately differentiated). The histologic classification was that of Jereb & Sandstedt (7). All the tumors showed large necrotic areas with vital tumor tissue generally present only in the periphery.

The effect of preoperative chemotherapy treatment on the tumor was well demonstrated at the sequential angiographic studies. The degree of reduction of blood supply indicated an extensive devitalisation of tumor tissue. Histologic examination confirmed that all tumors showed widespread necrosis. The effect on the tumor was independent of its degree of differentiation. In the two tumors where angiography demonstrated vascular supply from lumbar arteries pericapsular growth could be shown. The angiography after chemotherapy demonstrated in addition to the regression of the tumor tissue considerably more renal parenchyma than before treatment. This might be due to a recovery of the renal parenchyma when the growing tumor begins to shrink.

The present preliminary report may suggest that preoperative radiotherapy can be changed to chemotherapy alone in the treatment of Wilms tumor.

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REFERENCES

- 1 Cassady J D, Tefft M, Filler R M, Jaffe N & Hellman H. Considerations in the relation therapy of Wilms tumor. *Cancer* 37: 598 1973.
- 2 D'Angio G J, Beckwith J B, Bishop H C, Breslow N, Evans A, Goodwin W, King L, Pickett L, Sinks L F, Sutow W & Wolff J. The National Wilms Tumor Study: a progress report. *Proc Natl Cancer Conf* 7: 627 1973.
- 3 D'Angio G J, Beckwith J B, Bishop H C, Breslow N, Feigl P, Evans A, Goodwin W, Pickett L, Sinks L F, Sutow W, Tefft M & Wolff J. The National Wilms Tumor Study (NTWS)—Preliminary results. *Proc Am Assoc Cancer Res* 15: 1974 (Abstr).
- 4 D'Angio G J, Evans A E, Breslow N, Beckwith J B, Bishop H, Feigl P, Goodwin W, Leape L, Sinks L F, Sutow W, Tefft M & Wolff J. The treatment of Wilms tumor. *Cancer* 38: 633 1976.
- 5 Farber S. Chemotherapy in the treatment of leukemia and Wilms tumor. *JAMA* 198: 876 1966.
- 6 Jenkin D & Sonley M. Wilms tumor—Treatment results 1969–73. Implications for the future. *Proc Am Soc Clin Oncol* 16: 239 1975.
- 7 Jereb B & Sandstedt B. Structure and size versus prognosis in nephroblastoma. *Cancer* 31: 1471 1973.
- 8 Lemerle J, Voute P A, Tournade M F, Delamarre J F M, Jereb B, Åhström L, Flamant R & Gerard Marchant R. Preoperative versus postoperative radiotherapy: single versus multiple courses of actinomycin D in the treatment of Wilms tumor. *Cancer* 38: 647 1976.
- 9 Sullivan M P, Sutow W, Cangie A & Taylor G. Vincristine sulfate in management of Wilms tumor. Replacement of preoperative irradiation by chemotherapy. *JAMA* 207: 381 1967.

CASE REPORT

PNEUMONIA ASSOCIATED WITH *CHLAMYDIA TRACHOMATIS* INFECTION IN AN INFANT

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ABSTRACT Hallberg A Mårdh P A Persson K and Ripa T (Departments of Paediatrics and Virology Malmö General Hospital and Department of Medical Microbiology University Hospital Lund Sweden) Pneumonia associated with *Chlamydia trachomatis* infection in an infant Acta Paediatr Scand 68 765 1979.—*Chlamydia trachomatis* was isolated from the epipharynx of a 10-week-old baby girl taken ill with pneumonia but without signs of conjunctivitis The infant developed specific antibodies to the organism The course of the pneumonia was protracted with cough and tachypnea The baby who was afebrile improved on antibiotic therapy but pulmonary infiltrates persisted for several months To our knowledge this is the first case of pneumonia in an infant associated with *C trachomatis* infection reported elsewhere than North America

KEY WORDS Infant *Chlamydia trachomatis* pneumonia

During recent years it has become evident that *Chlamydia trachomatis* is a common cause of sexually transmitted diseases (15) and complications thereof such as acute epididymitis (5) and salpingitis (11)

C trachomatis may be transmitted from the mother to her baby resulting in conjunctivitis (12) and pneumonia (3) The first case of pneumonitis associated with *C trachomatis* infection was first described in 1975 by Schachter and colleagues (14) Since then a number of reports have appeared (2 3 7 8 9 10 15) all from North America describing chlamydia associated pneumonitis in newborns

In the present communication a case of pneumonia strongly suspected to be caused by *C trachomatis* is described in a Swedish infant

CASE HISTORY

A 10-week-old girl was admitted to the Department of Paediatrics Malmö General Hospital because of wheezing and cough persisting for 2 weeks She was in good condition and afebrile A diagnosis of obstructive bronchitis was recorded Two weeks later she was brought

back with symptoms of tachypnea cough and wheezing but was still afebrile A pulmonary radiograph revealed diffuse interstitial and patchy alveolar infiltrates She was given ampicillin (50 mg/kg/day) for 6 days and the symptoms regressed

C trachomatis was isolated from the epipharynx by means of an isolation technique described elsewhere (13) Tests for *Bordetella pertussis* and cytomegalovirus proved negative No antibodies to cytomegalovirus respiratory syncytial virus or to *Mycoplasma pneumoniae* were found

By the time the result of the culture for chlamydia was available she had improved and another pulmonary radiograph showed only a few remaining infiltrates Nevertheless erythromycin (60 mg/kg/day) was given for 2 weeks One week later the infant again had diffuse and widespread lung infiltrates and erythromycin was given for a further 2 weeks One week after the conclusion of this treatment the patient began to cough again and the lung infiltrates had progressed Trimethoprim/sulphamethoxazole (trimethoprim 8 mg/kg/day and sulphamethoxazole 40 mg/kg/day) was then given for 2 weeks after which her symptoms and the infiltrates disappeared Five days after she had completed the treatment course the infant once again started to cough she had tachypnea and was wheezing She was then admitted to the Department of Paediatrics

C trachomatis could no longer be isolated from the epipharynx nor from rectum vagina or the conjunctivae There was no eosinophilia and the serum levels of IgG and IgM were within the normal range (Table 1) The first serum sample from the infant was obtained at the age of 5 1/2 months IgG (but not IgM) antibodies to *C trachoma*

Table 1 Results of some laboratory tests

NT = not tested

Parameter studied	Age (weeks)		
	12	17	22
S leukocytes ($\times 10^9/l$)	6.0	9.0	7.8
ESR (mm)	25	18	13
S eosinophils ($\times 10^9/l$)	NT	NT	187
P IgG (g/l)	NT	NT	7
P IgM (g/l)	NT	NT	0.8
Weight (g)	4 890	5 620	6 470
Rectal temp (°C)	37.2	NT	36.8

titres at a titre of 1:512 were demonstrated using a micro-immunofluorescence test (17).

Trimethoprim/sulphamethoxazole was given for a further 4 weeks. The treatment resulted in the disappearance of the symptoms but diffuse pulmonary infiltrates still persisted.

At delivery the mother had IgG antibodies against *C. trachomatis* at a titre of 1:16-1:32. Six months later the titre was 1:256 (IgG). At the time when the girl was admitted to the Paediatric Department *C. trachomatis* could not be isolated from the cervix/urethra of the mother. However 3 months later the organism was recovered from both these sites. *C. trachomatis* could not be isolated from the single urethral specimen obtained from the father.

DISCUSSION

Infants may after birth be colonized with *C. trachomatis* in the eyes and/or epipharynx as well as in the rectum and genital tract (3, 14, 15). Some colonized infants may develop conjunctivitis and/or pneumonia. Recently Schachter et al. (16) estimated that 14 out of 1000 newborns in San Francisco develop conjunctivitis due to an infection with *C. trachomatis*. The corresponding figure for pneumonia is 8. Harrison and co-workers (10) found *C. trachomatis* in the nasopharynx and/or a specific antibody response to the organism in 9 out of 30 infants with signs of pneumonitis and in one of the 28 matched controls. Beem & Saxton (3) reported that in 18 infants with *C. trachomatis* associated pneumonia only half had conjunctivitis. They also found that 10 of 11 infants with conjunctivitis but without lower respiratory tract illness had a positive culture for *C. trachomatis* from the nasopharynx (3).

C. trachomatis was isolated from the epipharynx

of our patient whereas cultures from both eyes proved negative. Two months after the isolation of *C. trachomatis* from the epipharynx the child still had signs of pneumonia. She then had IgG antibodies to *C. trachomatis* at a titre of 1:512. There is no reason to believe that the antibodies demonstrated in the infant originated from the mother as the level in the child was much higher than had been detected in the mother prior to birth. The high level of IgG antibodies in the child was found at the age of 5½ months when maternal antibodies would be virtually gone. The failure to detect IgM antibodies probably reflects the late timing of specimen collection.

Conclusive evidence of *C. trachomatis* as being the cause of pneumonitis in newborn infants is the isolation of the organism from open lung biopsies of children with pneumonitis who have developed high titres of specific antibodies to the organism (2, 8). In our case the organism was not isolated directly from the pulmonary lesions but circumstantial evidence strongly points to *C. trachomatis* as being the cause of the pneumonia. The clinical course was consistent with that of some of the earlier reported cases of *C. trachomatis* associated pneumonia in infants (3, 9, 10, 14, 15).

C. trachomatis has been recovered in several series of pregnant women in frequencies up to 12% (1, 6, 15). It has been estimated (1, 6, 15) that the risk of a child developing a clinical infection with *C. trachomatis* is at least 50% if borne by a woman infected with the organism in the lower genital tract. The first set of cultures for *C. trachomatis* from the mother in the case described were negative which however might be due to the fact that the specimens had been stored frozen prior to study.

Harrison et al. (10) found that eosinophilia and/or elevated serum levels of IgG and IgM occur in some newborns with pneumonitis caused by *C. trachomatis*. No serum specimens were available from our patient until she reached the age of 5½ months. However on this occasion the infant had no eosinophilia

and normal serum levels of IgG and IgM (Table 1)

The protracted course of the disease in our patient corresponds to some earlier described cases (2-3). It may be wondered whether the child was reinfected by her mother who received no treatment until late in the course of her baby's illness.

Beem & Saxon (4) administered sulphisoxazole (150 mg/kg/day) to some infants with pneumonia and *C. trachomatis* infection while erythromycin ethylsuccinate (40 mg/kg/day) was given to others. Four days after treatment with either of these antibiotics chlamydiae could no longer be recovered from the epipharynx of the patients but not until 3 weeks later did pulmonary radiographs appear normal. In other cases of *C. trachomatis* associated pneumonia (9) recovery after 3 months without the institution of antibiotic treatment has been described. However as some infants may be seriously ill systemic therapy with erythromycin or sulphonamides has been recommended (4-15). The infant described here was given erythromycin for two 2 week periods and trimethoprim/sulphamethoxazole for a further 2 and 4 weeks respectively. During treatment she improved but the symptoms reappeared and the pulmonary infiltrates progressed shortly after treatment was discontinued. This indicates that antibiotic treatment of infants with pneumonia caused by *C. trachomatis* should be administered over a comparatively long period of time.

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REFERENCES

1. Alexander E R, Chandler J W, Pfeiffer T A, Wang S P, English M & Holmes K K. Prospective study of perinatal *Chlamydia trachomatis* infection. In: Hobson D & K Holmes (eds) *Nongonococcal urethritis and related infections*. American Society for Microbiology, Washington D C 1977, p 148.
2. Arth C, von Schmidt B, Grossman M & Schachter J. Chlamydial pneumonitis. *J Pediatr* 93: 447 1978.
3. Beem M O & Saxon E M. Respiratory tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N Engl J Med* 296: 306 1977.
4. Beem M O & Saxon E M. Uncertainties in the treatment of Chlamydia infections in infants. *N Engl J Med* 296: 1174 1977.
5. Berger R E, Alexander E R, Monda G B, Ansell J, McCormick G & Holmes K K. *Chlamydia trachomatis* as a cause of acute idiopathic epididymitis. *N Engl J Med* 298: 301 1978.
6. Chandler J W, Alexander E R, Pfeiffer T A, Wang S P, Holmes K K & English M. Ophthalmia neonatorum associated with maternal chlamydial infections. *Trans Am Acad Ophthalmol Otolaryngol* 83: 302 1977.
7. Embil J A, Ozere R L & MacDonald S W. Chlamydia trachomatis and pneumonia in infants: report of two cases. *Canad Med Assoc J* 119: 1199 1978.
8. Frommelt G T, Bruhn F W & Schwartzman J D. Isolation of *Chlamydia trachomatis* from infant lung tissue. *N Engl J Med* 296: 1150 1977.
9. Hammerschlag M R. Chlamydial pneumonia in infants. *N Engl J Med* 298: 1083 1978.
10. Harrison H R, English M G, Lee C K & Alexander E R. Chlamydia trachomatis infant pneumonitis. *N Engl J Med* 298: 70 1978.
11. Mårdh P A, Ripa K T, Svensson L & Westrom L. Chlamydia trachomatis infection of the fallopian tubes in patients with acute salpingitis. *N Engl J Med* 296: 1377 1977.
12. Rees E, Tait A, Hobson D & Johnson F W A. Chlamydia in relation to cervical infection and pelvic inflammatory disease. In: D Hobson & K K Holmes (eds) *Nongonococcal urethritis and related infections*. American Society for Microbiology, Washington D C 1977, p 67.
13. Richmond H J. Isolation of chlamydia subgroup A (*Chlamydia trachomatis*) in irradiated McCoy cells. *Medical Laboratory Technology* 31: 7 1974.
14. Schachter J, Lum L, Gooding C A & Ostler B. Pneumonitis following inclusion blennorrhoea. *J Pediatr* 87: 779 1975.
15. Schachter J. Chlamydia infections. *N Engl J Med* 298: 478, 490 and 540 1978.
16. Schachter J, Grossman M, Holt J, Sweet R & Mills J. Prospective study on chlamydial infection in the newborn. 18th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, Ga 1978. Abstract No 477.
17. Trehan J D, Darougar S & Jones R. Modification of the micro-immunofluorescence test to provide a routine serodiagnostic test for chlamydial infection. *J Clin Pathol* 30: 510 1977.

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Table 1 Results of some laboratory tests

NT = not tested

Parameter studied	Age(weeks)		
	12	17	22
S Leukocytes ($\times 10^9/l$)	6.0	9.0	7.8
ESR (mm)	25	18	13
S Eosinophils ($\times 10^9/l$)	NT	NT	187
P IgG (g/l)	NT	NT	7
P IgM (g/l)	NT	NT	0.8
Weight (g)	4 890	5 670	6 470
Rectal temp (°C)	37.2	NT	36.8

tis at a titre of 1:512 were demonstrated using a micro-immunofluorescence test (17).

Trimethoprim/sulphamethoxazole was given for a further 4 weeks. The treatment resulted in the disappearance of the symptoms but diffuse pulmonary infiltrates still persisted.

At delivery the mother had IgG antibodies against *C. trachomatis* at a titre of 1:16–1:32. Six months later the titre was 1:256 (IgG). At the time when the girl was admitted to the Paediatric Department *C. trachomatis* could not be isolated from the cervix/urethra of the mother. However, 3 months later the organism was recovered from both these sites. *C. trachomatis* could not be isolated from the single urethral specimen obtained from the father.

DISCUSSION

Infants may after birth be colonized with *C. trachomatis* in the eyes and/or epipharynx as well as in the rectum and genital tract (3, 14, 15). Some colonized infants may develop conjunctivitis and/or pneumonia. Recently Schachter et al. (16) estimated that 14 out of 1000 newborns in San Francisco develop conjunctivitis due to an infection with *C. trachomatis*. The corresponding figure for pneumonia is 8. Harrison and co-workers (10) found *C. trachomatis* in the nasopharynx and/or a specific antibody response to the organism in 9 out of 30 infants with signs of pneumonitis and in one of the 28 matched controls. Beem & Saxon (3) reported that in 18 infants with *C. trachomatis* associated pneumonia only half had conjunctivitis. They also found that 10 of 11 infants with conjunctivitis but without lower respiratory tract illness had a positive culture for *C. trachomatis* from the nasopharynx (3).

C. trachomatis was isolated from the epi-

pharynx of our patient whereas cultures from both eyes proved negative. Two months after the isolation of *C. trachomatis* from the epipharynx the child still had signs of pneumonia. She then had IgG antibodies to *C. trachomatis* at a titre of 1:512. There is no reason to believe that the antibodies demonstrated in the infant originated from the mother as the level in the child was much higher than had been detected in the mother prior to birth. The high level of IgG antibodies in the child was found at the age of 5½ months when maternal antibodies would be virtually gone. The failure to detect IgM antibodies probably reflects the late timing of specimen collection.

Conclusive evidence of *C. trachomatis* as being the cause of pneumonitis in newborn infants is the isolation of the organism from open lung biopsies of children with pneumonitis who have developed high titres of specific antibodies to the organism (2, 8). In our case the organism was not isolated directly from the pulmonary lesions but circumstantial evidence strongly points to *C. trachomatis* as being the cause of the pneumonia. The clinical course was consistent with that of some of the earlier reported cases of *C. trachomatis* associated pneumonia in infants (3, 9, 10, 14, 15).

C. trachomatis has been recovered in several series of pregnant women in frequencies up to 12% (1, 6, 15). It has been estimated (1, 6, 15) that the risk of a child developing a clinical infection with *C. trachomatis* is at least 50% if borne by a woman infected with the organism in the lower genital tract. The first set of cultures for *C. trachomatis* from the mother in the case described were negative which however might be due to the fact that the specimens had been stored frozen prior to study.

Harrison et al. (10) found that eosinophilia and/or elevated serum levels of IgG and IgM occur in some newborns with pneumonitis caused by *C. trachomatis*. No serum specimens were available from our patient until she reached the age of 5½ months. However, on this occasion the infant had no eosinophilia.

CASE REPORT

THE 49XXXX SYNDROME

Report of a Case with 48XXXX/49XXXX Mosaicism

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ABSTRACT Cirillo Silengo M, Davi G F and Franceschini P (the Clinical Genetics Center First Paediatric Clinic University of Turin Turin Italy) The 49XXXX syndrome Acta Paediatr Scand 68 769 1979.—A patient with 48XXXX/49XXXX mosaicism is presented. Clinical findings include severe growth and developmental retardation hyper telorism mongoloid slant of the palpebral fissures clinodactyly of the fifth fingers retarded bone age and radio-ulnar synostosis. The findings are similar to those of the cases with a pent X chromosome complement already described and are also similar to the signs of the more common 49XXXXY syndrome of males. In both instances the dysmorphic features are less impressive than the mental retardation and the skeletal malformations. This report contributes to a better delineation of the 49XXXX syndrome. The possible mechanisms of the chromosomal aberration are discussed.

KEY WORDS Chromosomal aberration X polipomy malformation syndromes

Hitherto only 8 patients with a 49XXXX karyotype have been reported by Berger et al (1) Borbolla Vacher et al (2) Brody et al (3) Giovannucci Uzielli et al (6) Kaufman et al (9) Kesaree & Wooley (10) Larger Piet et al (11) Sergovich et al (13) and Yamada & Nenishi (14).

Two 48XXXX/49XXXX mosaics were described by Cooke et al (4) and by Dalla Piccola & Pistocchi (5). The patient described by Gordon & Paulsen (7) had a complex mosaicism with four cell lines (XO/XX/XXY/XXXX).

We describe here a 14-month old female with a 48XXXX/49XXXX mosaicism who has clinical and radiological findings similar to those of the previously described cases.

CASE REPORT

R M Female age 14 months

Both parents are 3 years old and healthy. A 7 year-old brother is normal. She was the full term product of an un-
-y Delivery was accomplished by

cesarean section because of uterine inertia. Birth weight 1900 g. She was hypertonic as a newborn and failed to thrive. The psychomotor development has been delayed.

Physical examination

Weight 5300 g height 80 cm head circumference 47 cm. She has a peculiar facies with frontal bossing hyper telorism upward slant of the palpebral fissures and simple pattern ears (Fig 1).

She has marked clinodactyly of the fifth fingers. Dermoglyphics show bilateral displaced axial triradii and hypotenar loops. Because of the extreme dermal ridge hypoplasia the fingerprint pattern could not be evaluated.

She has hypotonia and joint hyperflexibility. Her development is severely retarded: she has poor head control cannot sit up stand walk nor talk.

Laboratory Studies

Routine investigations are within normal limits. The radiological findings are marked hypoplasia of the middle phalanx of the fifth fingers producing clinodactyly (Fig 2) proximal radio-ulnar synostosis (Fig 3) and increased iliac angle. The bone age is that of a newborn. The intra venous urogram is normal.

Cytogenetic Studies

The chromosomal analysis of the blood lymphocytes is consistent with a mosaicism with two cell lines: one with 48 chromosomes (25%) and one with 49 chromosomes

tion by a normal X bearing sperm. This hypothesis is based upon the data from the Xg blood group studies in three cases of X polysomy in whom the maternal derivation of the Xs was demonstrated and mosaicism was excluded (8-12). In our patient who has 48XXXX/49XXXXX cell lines one X chromosome could have been lost in a mitotic division of an originally 49XXXXX zygote. The presence of only two cell lines is against the hypothesis of repeated non-disjunctional events in mitotic divisions of a normal XX or a XXX zygote. This is probably the more reasonable interpretation of the patient reported by Gordon & Paulsen (7) who had four cell lines with a XO/XX/XXX/XXXXX mosaicism.

In our case the Xg blood group was not informative. On the other hand those studies when contributory allow us to confirm or to disprove the paternal contribution of X chromosomes but do not demonstrate whether the non-disjunctional events occur in meiosis post-zygotic mitosis or both. The presence of multiple cell lines seems a better criterion by which to ascertain the mitotic origin of a non-disjunctional error.

REFERENCES

- Berger R, Loewe Lyon S, Derre J & Ortiz M A. Syndrome 49 XXXXX. *Ann Pediatr* 70: 965 1973.
- Borbolla Vacher L, Vazquez Rios B, Pozo Lauzan D & Alvarez Mesa M. Syndrome 49,XXXXX. Presentacion de un caso. *Rev Cub Pediatr* 47: 4 1975.
- Brody J, Fitzgerald M C & Spies A. A female child with five X chromosomes. *J Pediatr* 70: 105 1967.
- Cooke H, Blanck J A & Curtiss J J. Comparative clinical studies and X chromosome behaviour in a case of XXXX/XXXXX mosaicism. *J Med Genet* 9: 235 1972.
- Dallapiccola B & Pistocchia G F. Alterazioni scheletriche in un caso di rara aberrazione cromosomica (paziente a corredo cromosomico 48XXXX/49XXXXX) studio comparativo con la sindrome 49XXXXX e la sindrome 49XXXXX. *Radiol Med* 54: 737 1968.
- Giovannucci Uzielli M L, Torricelli F, Salvatori Q, Consumi I, Donzelli G & Seminara S. Corredo cromosomico 49 XXXXX in una bambina con ipoevolutismo psicofisico. *Minerva Pediatr* 27: 2270 1975.
- Gordon D L & Paulsen C A. Premature menopause in XO/XX/XXX/XXXXX mosaicism. *Am J Obstet Gynecol* 97: 81 1967.
- Grouchy de J, Brissaud H, Richardet J M, Repesse G, Sanger R, Race R R, Salmon C & Salmon D. Syndrome 48 XXXX chez une enfant de six ans. Transmission anormale du groupe Xg. *Ann Genet* 11: 170 1968.
- Kaufman L, Sekhon G S, Brazy J E, Sivakoff M C & Hatahet G. 49XXXXX syndrome in a neonate. *Birth Defects* 11 (5): 333 1975.
- Kesaree L & Wooley P V. A phenotypic female with 49 chromosomes presumably XXXXX. *J Pediatr* 63: 1099 1963.
- Larger P, A, Bertholot J & Ement I. Une observation du syndrome 49 XXXXX. *Nouv Presse Med* 1: 467 1972.
- Lewis F J W, Froland A, Sanger R & Race R R. Source of the X chromosome in two XXXXY males. *Lancet* ii: 1099 1964.
- Sergovich F, Uilenber C & Pozsoni J. The 49 XXXXX chromosome constitution similarly to the 49 XXXXY condition. *J Pediatr* 78: 285 1971.
- Yamada Y, Nishish S, Penta X (49XXXXX) chromosome constitution: a case report. *Jap J Hum Genet* 16: 15 1971.

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Fig. 1 Patient's phenotype

(75%) The extra chromosomes were identified as λ s with Q bands by fluorescence using quinacrine and with G bands by trypsin using Giemsa.

The buccal smear is positive in 84% of the examined cells. 15% of the cells have a single Barr body. Two, three and four Barr bodies are present in 23% of the cells respectively. Skin fibroblasts have the same chromosomal pattern. Thus the patient is a 48 λ XXX/49XXXXX mosaic. The karyotype of both parents is normal. The X_b blood group study is non-contributory.

DISCUSSION

On comparing the literature data with ours, the existence of a penta X syndrome seems acceptable.

Constant findings are severe psychomotor retardation, growth failure, peculiar facies with coarse features, hypertelorism, mongoloid slant of the palpebral fissures, clinodactyly of the fifth fingers. Half of the patients have retarded bone age and radio ulnar synostosis. In three cases (2, 5, 13) a number of

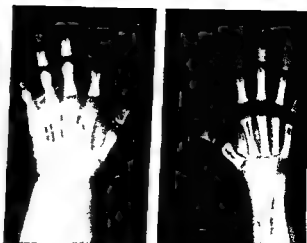


Fig. 2 Hands X rays illustrating the clinodactyly of the fifth fingers

other skeletal malformations similar to those found in the 49XXXXY are recorded.

The clinical similarity of males and females with 49 chromosomes and to a lesser extent females with 48XXXX karyotype draws attention to the possible effect of constitutive heterochromatin overdosage in skeletal and mental development as well as in phenotype expression. The marked prevalence of 49XXXXY males over 49XXXXX and 48XXXX females is possibly explained as a bias of ascertainment due to the genital anomalies in males.

The simplest explanation for a 49XXXXX karyotype is a double meiotic non-disjunction in the mother with the production of a 26XXXX ovum and its subsequent fecunda-



Fig. 3 Elbow X rays: bilateral radio ulnar synostosis

tion by a normal X bearing sperm. This hypothesis is based upon the data from the Xg blood group studies in three cases of X polysomy in whom the maternal derivation of the Xs was demonstrated and mosaicism was excluded (8-12). In our patient who has 48XXXX/49XXXXX cell lines one X chromosome could have been lost in a mitotic division of an originally 49XXXXX zygote. The presence of only two cell lines is against the hypothesis of repeated non-disjunctional events in mitotic divisions of a normal XX or a XXX zygote. This is probably the more reasonable interpretation of the patient reported by Gordon & Paulsen (7) who had four cell lines with a XO/XX/XXX/XXXXX mosaicism.

In our case the Xg blood group was not informative. On the other hand those studies when contributory allow us to confirm or to improve the paternal contribution of X chromosomes but do not demonstrate whether the non-disjunctional events occur in meiosis or in zygotic mitosis or both. The presence of multiple cell lines seems a better criterion by which to ascertain the mitotic origin of a non-disjunctional error.

REFERENCES

- Berger R, Loewe Lyon S, Derre J & Ortiz M A. Syndrome 49XXXXX. *Ann Pediatr* 70: 965 1973.
- Bortolla Vacher L, Vasquez Rios B, Pozo Lauzan D & Alvarez Mesa S. Syndrome 49XXXXX. Presentación de un caso. *Rev Cub Pediatr* 47: 4 1975.
- Brody J, Fitzgerald M C & Spies A S. A female child with five X chromosomes. *J Pediatr* 70: 105 1967.
- Cooke P, Black J A & Curtid D J. Comparative clinical studies and X chromosome behaviour in a case of XXXXX/XXXXX mosaicism. *J Med Genet* 9: 735 1972.
- Dallapiccola B & Pistocchi G F. Alterazioni scheletriche in un caso di rara aberrazione cromosomica (paziente a corredo cromosomico 48XXXX/49XXXXX) studio comparativo con la sindrome 49XXXXX e la sindrome 49XXXXX. *Radiol Med* 54: 737 1968.
- Giovannucci Uzielli M L, Torricelli F, Salvatori Q, Consumi I, Donzelli G & Seminara S. Corredo cromosomico a 49XXXXX in una bambina con ipoevolutismo psicofisico. *Minerva Pediatr* 27: 2720 1975.
- Gordon D L & Paulsen C A. Premature menopause in XO/XX/XXX/XXXXX mosaicism. *Am J Obstet Gynecol* 97: 85 1967.
- Grouchy de J, Brissaud H E, Richardet J M, Repesse C, Sanger R, Race R, Salmon C & Salmon D. Syndrome 48XXXX chez une enfant de six ans. Transmission anormale du groupe Xg. *Ann Genet* 11: 1-0 1968.
- Kaufman L, Sekhon G S, Brazy J E, Sivakoff M C & Hatahet G. 49XXXXX syndrome in a neonate. *Birth Defects* 11 (5): 333 1975.
- Kesaree L & Wooley P V. A phenotypic female with 49 chromosomes presumably XXXXX. *J Pediatr* 63: 1099 1963.
- Larger Piet A, Bertholot J & Ement I. Une observation du syndrome 49XXXXX. *Nouv Presse Med* 1: 467 1972.
- Lewis F J W, Froland A, Sanger R & Race R R. Source of the X chromosome in two XXXXY males. *Lancet* ii: 1099 1964.
- Sergovich F, Uitenber C & Pozsoni J. The 49XXXXX chromosome constitution similarity to the 49XXXXY condition. *J Pediatr* 88: 285 1971.
- Yamada Y, Nishiyoshi S, Penta X (49XXXXX) chromosome constitution: a case report. *Jap J Hum Genet* 16: 15 1971.

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CASE REPORT

NEONATAL RESPIRATORY INSUFFICIENCY DUE TO CENTRONUCLEAR MYOPATHY

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ABSTRACT Reitter W Mortier W and Wille L (Children's Hospital University of Heidelberg Children's Hospital University of Düsseldorf FRG) Neonatal respiratory insufficiency due to centronuclear myopathy. *Acta Paediatr Scand* 68 773 1979. —Two neonates showing generalized hypotonia weakness of limbs trunk and oral musculature died because of muscular respiratory distress. The diagnosis of centronuclear (or myotubular) myopathy was established by histological and histochemical techniques. The genetic situation and routine laboratory data including electromyography were compared with similar cases in the literature. Findings were inconclusive with respect to this diagnosis. These results indicate the need for a muscle biopsy and the use of histochemical stainings and/or electronmicroscopical investigation for a proper diagnosis in hypotonic newborns under respiratory distress after exclusion of etiologies other than neuromuscular diseases. Still the diagnosis of centronuclear myopathy in a neonate does not allow a precise prognosis. Increased awareness of this disorder and adequate diagnostic workup is needed in order to extend our understanding and to clarify the prognosis.

KEY WORDS Neonates respiratory insufficiency centronuclear myopathy

Severe muscular hypotonia and weakness may cause respiratory insufficiency and in some instances death during the early neonatal period. This is known from cases of spinal muscular atrophy (12) and neonatal myasthenia gravis. There seems to be an increasing awareness of a similar clinical course in centronuclear or myotubular myopathy, one of the congenital structural myopathies, which in general allows a good prognosis (17). Since our first case report (18), a second newborn was diagnosed in our hospital and others reported on rather similar clinical and histopathological characteristics (17). In order to help in delineating common features of this malignant variant of centronuclear myopathy, we shall detail our two cases.

III para III. Her first pregnancy and delivery were uneventful; however, the floppy male newborn died at the age of 11 days due to respiratory insufficiency. The second pregnancy resulted in a healthy boy who developed



Fig 1 Case 1 roentgenogram of the thorax showing slender ribs and diaphragmatic eventration on the right side.

CASE REPORTS

Case 1. A B is the third child of healthy non-consanguineous parents. The mother was a 36-year-old gravida

Presented at the meeting of the Deutsche Gesellschaft für Kinderheilkunde, Köln, September 1976.



Fig 2 Case 1 hypotonic posture unchanged throughout the clinical course

normally. During the third pregnancy there were reduced fetal movements.

The male infant weighing 2650 g was delivered by caesarian section during the 38th week of gestation. Apgar score was six at one minute and seven at five minutes. Because of insufficient breathing the newborn required immediate intubation and assisted ventilation. On X-ray only small ribs and a diaphragmatic eventration on the right side were noted (Fig. 1). ECG was according to age. During the clinical course constant frog position with extreme generalized muscular hypotonia and sparse spontaneous movements of the limbs and the trunk were noticed (Fig. 2). Deep tendon reflexes could hardly be elicited. Laboratory data: Serum aldolase and creatine

kinase (CK) were within normal limits. Aldolase 8 I U/l (=135 nkat/l), CK 83 U/l (=1383 nkat/l).

An EMG of the anterior tibial muscle and the thenar on the right side corresponded more closely to a neurogenic lesion. The motor nerve conduction velocities of the superficial peroneal and the posterior tibial nerve on the right side were normal for his age (29 and 76 m/s respectively).

Two attempts to wean the newborn off the respirator failed and he died on his sixth day.

Postmortem examination revealed hypoplasia of the skeletal musculature including the diaphragm. On electron microscopy a few myelin sheaths of peripheral nerves appeared discontinuous. Histologically the striated muscles of different areas including the tongue showed increased variability in fiber size (Fig. 3). There was an abnormally high proportion of small fibers. About 40% of the muscle fiber nuclei were centrally located and often surrounded by a zone devoid of myofibrils.

This was seen more frequently in the fibers with small diameters. The intercostal muscles and the tongue showed these myotubular structures to a higher degree than the other muscle groups.¹ PAS positive material was increased (Fig. 4). Histochemical stainings (NADH-GPDH and ATPase reactions) (Fig. 5) confirmed the histological diagnosis of centronuclear myopathy. In addition type II fibers outnumbered type I fibers (67 versus 33%). Type III fibers were almost lacking.

¹ These postmortem studies were done by PD Dr H. P. Schmitt and Dr B. Volk, Neuropathological Institute, University of Heidelberg (Director Prof. Dr W. Ule). The same applies to case 2.

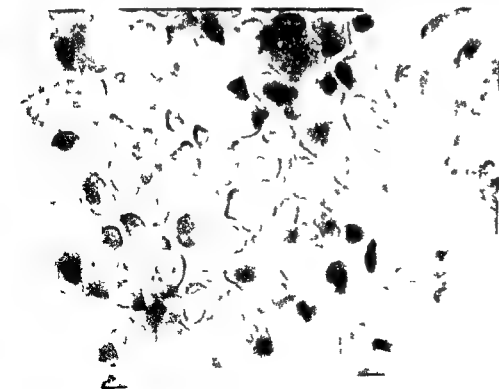


Fig 3 Variation in fiber diameter, centrally placed nuclei in the smaller fibers, cross section. Trichrome $\times 250$.

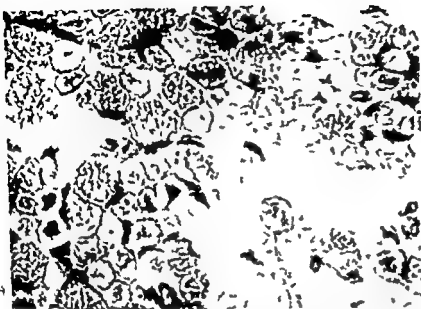


Fig 4 Increased PAS positive material PAS $\times 250$

Case 2 N 5 first child of healthy not related parents. The mother was a 30-year-old gravida I para I. During her pregnancy hydramnion and intrauterine dystrophy were suspected. Following premature rupture of membranes a female newborn weighing 2770 g was delivered spontaneously at term. Apgar notes were two at one minute and three at ten minutes. Immediately after birth intubation and ventilatory assistance were necessary because of poor breathing efforts. On admission to the newborn intensive care unit chest X rays showed strikingly thin end small ribs and clavicles. Clinically there were poor spontaneous movements of the hypotonic limbs de-

creased tendon reflexes, a lack of head control and no fasciculation. Transient improvement of spontaneous breathing allowed weaning off the respirator and extubation on the third day. However, due to progressive shallowness of breathing the newborn had to be reintubated and ventilated again.

Laboratory investigation: Serum levels of SGOT 60 U/l (≈ 1000 nkat/l), CK 170 U/l (≈ 7000 nkat/l) and aldolase 19.6 U/l (≈ 376.7 nkat/l) were elevated. The level of SGPT (36 U/l-600 12 nkat/l) was normal. EMG data were obtained from the right rectus femoris and the biceps brachii muscle and had to be limited to a brief

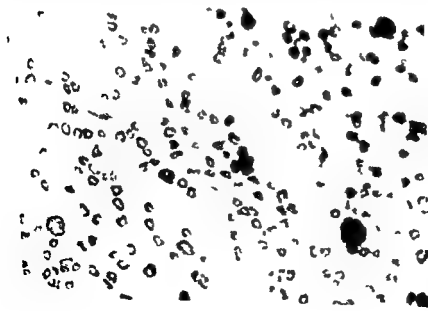


Fig 5 Absence of ATPase activity in central areas of many fibers. ATPase preincubated at pH 4.6 $\times 100$



Fig. 2 Case 1 hypotonic posture unchanged throughout the clinical course

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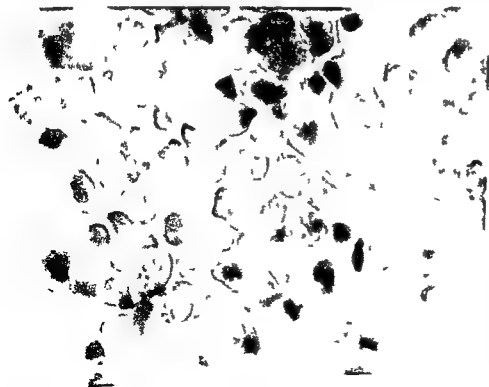


Fig. 3 Variation in fiber diameter centrally placed nuclei in the smaller fibers cross section. Trichrome

cular disease with pre or perinatal onset (Table 1). Reviewing comparable cases in the literature (8, 17, 22) symptoms include bilateral ptosis, facial diplegia and external ocular muscle weakness. None of these signs were seen in our two cases. In clinical differential diagnosis some features of other neuromuscular disorders not reported from known cases of neonatal centronuclear myopathy might be helpful (Table 2).

Probably all newborns coming to medical attention because of respiratory distress undergo X-ray examination of the chest. The striking anomaly in both of our children was the underness of the ribs and clavicles (Fig. 1). This phenomenon is known to be associated with other conditions like myotonic dystrophy. Our own experience relates this anomaly to neuromuscular diseases with prenatal onset.

Determination of muscle enzyme activities seems to be inconclusive for the diagnosis of centronuclear myopathy. Raju (17) reported normal CK level, one child described here had a borderline and the other a moderately elevated CK activity.

Electrophysiological data are not contributory (3, 6, 8, 17). Only the histological, histochemical and electromicroscopical characteristics of striated muscle can determine the diagnosis of centronuclear myopathy. The specific microscopic findings are described elsewhere in great detail (3, 5, 7, 9, 14, 16, 21).

However, the demonstration of structures resembling myotubes in the patient's muscle biopsy with or without a major degree of fiber size disproportion still does not provide a precise prognosis. The degree of involvement of different muscles and muscle groups appears to vary (1) as does the distribution within the group of muscle fibers (22). One of those authors judging centronuclear myopathy to be a progressive dystrophy primarily limited to type I fibers (2, 3) points to the difference of type I fibers with central nuclei in a gastrocnemius muscle biopsy compared to 27% in the same muscle at autopsy 17 years later (2).

Therefore quantitative histological results of muscle biopsies will not elucidate the expected course of disease for an individual. More precision in prognosis is needed for decisions about long term care of affected newborns and for genetic counseling. A greater number of diagnosed cases might help to delineate common phenomena. The short interval elapsing between the cases observed by our group and others allows the assumption that a higher incidence of centronuclear myopathy in neonates exists as does an increasing frequency of a proper diagnosis due to awareness of the condition.

REFERENCES

- 1 Barth P G, van Wyngaarden H K & Bethlem J. X linked myotubular myopathy with fatal neonatal asphyxia. *Neurology* 25: 511, 1975.
- 2 Bradley W G, Price H L & Watanabe C K. Familial centronuclear myopathy. *J Neurol Neurosurg Psychiatry* 33: 687, 1970.
- 3 Campbell M J, Rebeiz J J & Walton J N. Myotubular centronuclear or pericentronuclear myopathy? *J Neurol Sci* 8: 475, 1969.
- 4 Chassevent J, Sauvegrain J, Besson Leaud M & Kalifa G. Myotonic dystrophy (Steiner's disease) in the neonate. *Radiology* 127: 747, 1978.
- 5 Coleman H F, Thompson L R, Niehus A W, Munsat T L & Pearson C M. Histochemical investigation of myotubular myopathy. *Arch Pathol* 86: 365, 1968.
- 6 Coquet M, Vital C, Batin J, Hehunstre J P & Hedreville M A. Un cas de myopathie congénitale associant une hypotrophie des fibres de type I des ventricles du myocarde et une atrophie myofibrillaire focale. *Arch Anat Cytol Pathol* 24: 275, 1976.
- 7 Dubowitz V & Brooke M H. *Muscle biopsy: A modern approach*. W B Saunders Company Ltd, London, 1973, pp. 276-279.
- 8 Engel W K, Gold G N & Karpatis G. Type I fiber hypotrophy and central nuclei. *Arch Neurol* 18: 435, 1968.
- 9 Inokuchi T, Umezaki H & Santa T. A case of type I muscle fiber hypotrophy and internal nuclei. *J Neurol Neurosurg Psychiatry* 38: 475, 1975.
- 10 Karpatis G, Carpenter S & Nelson R F. Type I fiber atrophy and central nuclei: A rare familial neuromuscular disease. *J Neurol Sci* 10: 489, 1970.
- 11 Kinoshita M, Satoyoshi E & Matsuo N. Myotubular myopathy and type I fiber atrophy in a family. *J Neurol Sci* 26: 575, 1975.
- 12 Kyllerman M. Infantile spinal muscular atrophy (Morbus Werdnig-Hoffman) causing neonatal asphyxia. *Neuropediatrics* 8: 53, 1977.
- 13 McLeod J G, Baker De C W, Lethlean A K &

Table 1 *Symptoms in centronuclear myopathy causing neonatal respiratory distress*

History of poor fetal movements
Occasional hydramnion
Proper position and lacking head control due to hypotonia
Decreased spontaneous movements due to extreme weakness
Weak cry sucking swallowing
Insufficient breathing
Diminished or absent tendon reflexes

orientation. Motor nerve conduction velocities were not measured due to the child's poor condition. In the rectus femoris and the biceps muscle shortened potentials and an interference pattern with only slight contraction in the biceps muscle could point to a myopathic process. Following aspiration pneumonia developed and caused death on the fourth day of life.

Postmortem examination. Pale appearance of generally hypotrophic skeletal musculature. Microscopy: the striated muscle fibers showed increased variability in diameter (5 to 14 μ , normal for age 8 to 10 μ). Up to 50% of the nuclei were centrally located. They were frequently lacking in the central zones. The differentiation of the fiber types remained insecure. The ATPase reactions point to a fiber type II predominance.

The parents of both children were examined neurologically and electromyographically without pathological findings. The mother of the second child underwent muscle biopsy. The histological and histochemical results were within normal limits. Careful evaluation of the pedigrees of both children did not reveal any factors raising the suspicion of a neuromuscular disorder except with the deceased newborn in the family of case 1.

DISCUSSION

Generally the myopathies showing congenital structural anomalies and no histological evidence of inflammation or degeneration are judged to be benign in their clinical course. In most cases described hitherto clinical symptoms like muscle weakness and statomotor disablement led to a diagnostic workup beyond infancy. However all six patients of the family described by Wijngaarden et al (22) were floppy already in the newborn period and had temporary breathing difficulties. While in this family two patients died within the first three weeks of life due to respiratory insufficiency and/or secondary pulmonary infections, the second family observed by the

same authors (1) lost all children affected in the neonatal period. In both families a recessive X linked genetic transmission could be traced. Barth (1) assumes different alleles on the X chromosome for the explanation of the difference in severity. However for clinical decisions even a positive genetic background with clear mode of transmission does not seem very helpful. Besides autosomal dominant (10, 13, 14) and probable autosomal recessive (20) transmission is known for centronuclear myopathy. Therefore no common mode of genetic background can be shown so far for the severe clinical involvement. Furthermore the coincidence of structural myopathies with in one family (11) as well as within one patient (6, 19) has been reported. Thus an uneventful family history or the death of another newborn due to centronuclear myopathy does not provide precise information with respect to the individual prognosis of autosomal recessive inheritance or sporadic occurrence.

After excluding cardiac and pulmonary etiologies hypotonia with respiratory distress in a newborn should alert the physician to consider a structural myopathy regardless of the family history.

The clinical symptoms of a newborn severely affected with centronuclear myopathy hardly differ from those in any other neuro-

Table 2 *Symptoms of neuromuscular disease not reported from centronuclear myopathy in the neonatal period*

Symptom	Disease
Fasciculation	Infantile spinal muscular atrophy
Muscle wasting	Infantile spinal muscular atrophy, syndrom of muscle fiber disproportion
Contractures	Congenital muscular dystrophy, congenital unspecific myopathy (benign or progressive), arthrogryposis of different etiology
Myotonic discharges in EMG	Myotonic dystrophy

CASE REPORT

RENAL TUBULAR ACIDOSIS ASSOCIATED WITH TYPE III GLYCOGENOSIS

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ABSTRACT Cohen J and Friedman M (Department of Paediatrics Whittington Hospital London England) Renal tubular acidosis associated with type III glycogenosis. *Acta Paediatr Scand* 68 779 1979. —Two children who presented with severe failure to thrive were found to have Type III glycogen storage disease. They both also had defects of tubular acidification an association not previously described. The nature of the tubular lesion is characterized and the explanation and therapeutic implications are discussed.

KEY WORDS Type III glycogen storage disease renal tubular acidosis

Renal tubular acidosis has been described as a rare association of Type I glycogen storage disease (GSD) (4) but not previously with any of the other types. We describe two cases of Type III glycogenosis in each of whom a defect of urinary acidification was found.

CASE REPORTS

Case 1

This male infant was born in Doha, birth weight 3200 g after an uneventful pregnancy. He was well until one year of age when he was noted to be underweight and to have hepatomegaly. The father described episodes of pallor and sweating usually occurring before feeds. There had been no convulsions. His parents were first cousins and in good health. There were five siblings, all of whom were well.

On examination his weight was 6.6 kg (2.2 kg below the third percentile), his length was 70 cm (7 cm below the third percentile) and his head circumference 45.5 cm (which lay on the third percentile). The only other abnormal finding was a liver edge palpable 3 cm below the costal margin. He was developmentally normal. Investigations showed haemoglobin 137 g/l, urea 3.0 mmol/l, sodium 138 mmol/l, potassium 4.0 mmol/l, bicarbonate 16 mmol/l, chloride 107 mmol/l. Liver function tests, serum and urinary calcium and phosphate were all normal. There was no evidence of malabsorption. Routine urinalysis was negative. Plasma glucose after an overnight fast was 3.6 mmol/l and he had an appropriate response to glucagon, but during the course of his admission he had an episode similar to those described by his father during which his plasma glucose was less than 1 mmol/l. A liver

biopsy performed elsewhere had shown histological features compatible with a diagnosis of GSD but had not been submitted for enzyme histochemistry. Subsequent studies on leucocytes and a muscle biopsy showed him to have Type III GSD (Table 1). Further metabolic studies are shown in Table 2. Investigation of renal tubular function revealed an arterial pH of 7.37, bicarbonate 18 mmol/l with a simultaneous urine pH of 7.7. In response to an ammonium chloride load (0.1 g/kg) the urine pH did not fall below 5.38. An overnight water deprivation test was normal as was a urinary aminoacid chromatogram. Chest X-ray and an intravenous urogram were both normal.

Case 2

This thirteen month-old male infant was born in Abu Dhabi at term, birth weight 2700 g. At the age of three months he was observed to be small, a poor feeder and to have delayed motor development. Routine urinalysis showed heavy glycosuria in the presence of a normal plasma glucose. His parents were first cousins, were well and there were no siblings.

On examination his weight was 5.0 kg (3.5 kg below the third percentile), his length 60 cm (10 cm below the third percentile) and his head circumference 40 cm (4.5 cm below the third percentile). The liver edge was palpable 5 cm below the costal margin and his motor development was slightly retarded. The remainder of the examination was normal.

Initial investigations showed haemoglobin 116 g/l, urea 3.0 mmol/l, sodium 141 mmol/l, potassium 4.3 mmol/l, bicarbonate 16.5 mmol/l, chloride 110 mmol/l. Fasting plasma glucose 3.6 mmol/l. Liver function tests showed moderate elevation of serum hydroxybutyrate dehydrogenase to 530 IU/l (0-790) but were otherwise normal. Routine urinalysis showed 2⁺ glucose and was normal on microscopy. There was no evidence of malabsorption.

- Shorey C D Centronuclear myopathy with autosomal dominant inheritance *J Neurol Sci* 15 375 1972
- 14 Mortier W Michiels E Becker J & Gerhard I Centronucleäre Myopathie mit autosomal dominantem Erbgang *Hum Genet* 27 199 1975
- 15 Mortier W Besondere Probleme bei der elektrodiagnostischen Untersuchung von Kindern *J EEG EMG* 8 36 1977
- 16 Munsat T L Thompson L R & Coleman R F Centronuclear (myotubular) myopathy *Arch Neurol* 20 120 1969
- 17 Riju T N K Vidyasagar D Reyes M G & Chokroverty S Centronuclear myopathy in the newborn period causing severe respiratory distress *Pediatrics* 59 29 1977
- 18 Reitter B Stolz L Schmitt H P & Scheffner D Perinatale Form der myotubulären Myopathie in der Differentialdiagnose frühkindlicher neuromuskulärer Erkrankungen *Mschr Kinderheilk* 125 498 1977
- 19 Scelsi R Lanzi G Nespoli L & Poggi P Congenital non progressive myopathy with type II fibre atrophy and internal nuclei *Eur Neurol* 14 785 1976
- 20 Sher J H Rimmlowski A H Athanassiades T J & Aronson S M Familial centronuclear myopathy. A clinical and pathological study *Neurology* 17 727 1967
- 21 Spiro A J Shy G M & Gonatas N K Myotubular myopathy *Arch Neurol* 14 1 1966
- 22 Van Wijngaarden G K Fleury P Bethlem J & Meyer A E F H Familial myotubular myopathy *Neurology* 19 901 1969

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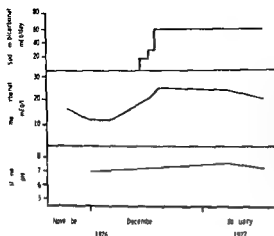


Fig 1 Case 1 Daily measurements of plasma bicarbonate and urinary pH during low dose oral bicarbonate therapy

clinical and biochemical features of Type III glycogenosis which previously have been well described (7). Both presented because of their failure to thrive and in the first case the history was typical of hypoglycaemic attacks. Although these classically occur in Type I GSD, fasting hypoglycaemia is well recognised in the debranching enzyme defect (1). Despite the hepatomegaly, mild elevation of the transaminases in the second patient was the only abnormality in liver function tests. Increased serum urate and blood lactate are only found in Type I GSD, probably as a consequence of prolonged hypoglycaemia. The lipoprotein abnormality found in the second case has also been previously described (7).

The definition and classification of renal tubular acidosis (RTA) has been discussed by Sebastian & Morris (6). They have identified three principal types of RTA and various hybrids, and it is clear from these descriptions that individual cases may demonstrate considerable overlap. Nevertheless, it is helpful to try and categorize as far as possible the two patients described here.

The first case demonstrated classical distal RTA. A mild systemic acidosis was present in the face of a strongly alkaline urine and in response to an ammonium chloride

load, the urine pH failed to fall below 5.2 on two separate occasions. It is difficult to exclude a small degree of bicarbonate wastage from the proximal tubule occurring concurrently, and although further studies (e.g. determination of the tubular maximum for bicarbonate) would help to clarify this, they were not thought to be appropriate to the management of this case. Subsequent treatment of this patient with low dose sodium bicarbonate (6 mEq/kg/day) effectively reversed the acidosis (Fig 1) and this is consistent with previous experience of treating distal RTA (8).

In the second case there was a more severe hyperchloraemic acidosis in conjunction with an acid urine. The additional finding of tubular proteinuria and persistent glycosuria suggested the presence of a partial Fanconi syndrome associated with a proximal bicarbonate wasting RTA.

This was tested by giving an oral bicarbonate load. Plasma bicarbonate prior to treatment was 10–15 mEq/l. Low dose treatment immediately elevated the plasma level to above the threshold for reabsorption, causing bicarbonate to spill into the urine. However, it was insufficient to maintain this effect and the plasma bicarbonate rapidly fell to pre-treatment levels, the urine becoming acid. Dou-

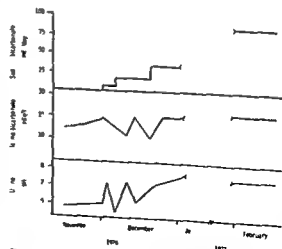


Fig 2 Case 2 Daily measurements of plasma bicarbonate and urinary pH during high dose oral bicarbonate therapy

Table 1 Summary of enzyme histochemistry confirming both patients to have Type III GSD

		Patient	Control	Normal range
Case I				
Whole blood glycogen	μg of glycogen ml^{-1} of whole blood	56.6		7-19
Leucocytes	Debranching system μmol glucose $\text{min}^{-1} \text{mg}^{-1}$ of protein Phosphorylase limit dextrin substrate	0.06		0.6-0.9
Muscle biopsy	Glycogen level	1.4%		<0.2%
	Debranching activity μmol glucose $\text{min}^{-1} \text{mg}^{-1}$ of tissue Phosphorylase limit dextrin substrate	0.011	0.66	
	α Schindinger substrate	0.07	0.17	
Case II				
Leucocytes	Debranching system μmol glucose formed $\text{min}^{-1} \text{mg}^{-1}$ of protein	0.13		0.6-0.9
Liver biopsy	Glycogen	12%		<4%
	Debranching system μmol glucose $\text{min}^{-1} \text{mg}^{-1}$ of tissue Phosphorylase limit dextrin substrate	0.05		0.2-0.6
Muscle biopsy	Glycogen	1.3%	0.17%	<0.2%
	Debranching system phosphorylase limit dextrin substrate	0.009	0.66	
	α Schindinger substrate	0.1	0.12	0.8-0.9

and the chest X ray was clear. Screening for glycogen storage disease was initially carried out on leucocytes and a diagnosis of Type III GSD later confirmed by percutaneous needle biopsy of the liver and muscle biopsy (Table 1). Subsequent metabolic studies are shown in Table 2.

Blood gas analysis showed a systemic acidosis: arterial pH 7.34, bicarbonate 12 mmol/l with a simultaneous urinary pH of 5.4. A urinary amino-acid chromatogram was normal and sugar chromatography confirmed glucose only to be present. Urinary protein electrophoresis demonstrated beta 2 microglobulin. There was no phosphaturia and no defect of tubular concentrating ability.

An intravenous urogram was normal. To clarify the nature of the renal tubular lesion a bicarbonate loading

test was carried out beginning with 2 mEq/kg of 8.4% sodium bicarbonate daily in divided doses by mouth. Daily simultaneous measurement of plasma bicarbonate and urine pH were made and the results are shown in Fig. 1.

DISCUSSION

Type III glycogen storage disease is caused by a relative deficiency of the debranching enzyme system (7) and in both these patients a specific enzyme assay revealed this deficiency. Both cases demonstrated a number of

Table 2 Details of further biochemical investigations

Case II had a mild hypertriglyceridaemia

Investigation	Normal range (mmol/l)	Case I	Case II
Serum uric acid	0.1-0.4	0.36	0.11
Blood lactate	1.0-1.78	1.88	1.33
Fasting serum cholesterol	3.6-6.7	3.4	5.2
Fasting serum triglyceride	0.2-1.6	1.0	2.1
Glucagon test			
plasma glucose	Fasting	3.6	3.6
	20 min after 1M glucagon	6.4	5.0

CASE REPORT

LOCALIZED SCLERODERMA FOLLOWING VARICELLA
IN A THREE YEAR OLD GIRL WITH IgA DEFICIENCY

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ABSTRACT Spierer Z, Ilie B, Pick I A and Yaron M (Departments of Pediatrics, Physical Medicine and Rheumatology, Pathology and the Section of Clinical Immunology, Rokach (Hadassah) and Ichilov Medical Center, Tel Aviv; Beilinson Medical Center, Petah Tiqva and the Sackler School of Medicine, Tel Aviv University, Israel). Localized scleroderma following varicella in a three year-old girl with IgA deficiency. *Acta Paediatr Scand* 68: 783, 1979.—A three year old girl with isolated IgA deficiency developed localized scleroderma immediately after varicella infection. Physiotherapy was started without any drug therapy. Only a minimal clinical improvement was achieved. The connection between IgA deficiency, viral infection and collagen diseases is discussed.

KEY WORDS IgA deficiency, isolated scleroderma, varicella

Systemic and localized scleroderma are rare diseases in children. Chazen et al. (4) reported in 1962 nineteen cases of localized scleroderma seen at the Children's Hospital Medical Center in Boston and at the Vanderbilt University Hospital's Pediatric Service between 1975 and 1960. We report here a case of localized scleroderma first noticed after varicella in a girl aged three who also has isolated IgA deficiency.

CASE REPORT

A B was born in November 1977 after normal gestation and delivery. She has a six year-old healthy sister and healthy parents. Her birth weight was 3000 g. She was not breast fed and developed normally. She was vaccinated against smallpox, tetanus, pertussis, polio and tuberculosis in due course and with no side effects. In March 1975 she had mumps. She had also suffered from two episodes of otitis media which required antibiotics but presented no serious problem. In January 1976 she developed varicella with fever up to 39°C and papular lesions over her entire body. Her mother reported that skin lesions were more numerous on her left upper extremity. A few days after the onset of varicella her mother noticed that the skin over her entire left upper extremity became tight and the child refrained from using her left arm.

Fluorination in February 1976 revealed a pleasant, alert

and intelligent girl. Her height was 95.5 cm and her weight was 13 kg. Changes typical for scleroderma were present in the skin of the left upper extremity. No changes in the skin of other parts of the body were noticed. The skin of the entire left upper extremity was shiny and tightly bound to tissues below. There was no dysphagia.

Laboratory examinations revealed hemoglobin 176 g/l, white count $8.7 \times 10^9/l$ with a normal differential, sedimentation rate 50/90 (Westergren), total protein 74 g/l, albumin 43 g/l, globulin 31 g/l. Protein paper electrophoresis showed albumin 57%, alpha 1 globulin 3%, alpha 2 globulin 7%, beta globulin 8% and gamma globulin 25%. IgG was within the normal range in the patient's serum as well as in the serum of her father, mother and sister. IgA was absent in the serum and saliva of the patient and of her father but was normal in that of her mother and sister. Antinuclear factor was present in the serum (antititer is 25%). LE preparation, Latex and Rose-Waaler tests were negative. Total hemolytic complement was 330 units/l (four normal ranges 400-800). C₃ was 56 mg/100 ml (four normal ranges 55-170). C was 19.2 mg/100 ml (four normal ranges 20-50). Mantoux test was negative. Serum transaminase, creatine phosphokinase and aldolase levels were normal. Electromyographic examination was normal. A biopsy from the left deltoid muscle was performed in February 1976 and showed pathological changes compatible with scleroderma in the skin, subcutaneous tissue and adjacent muscle (Fig. 1a and b).

A diagnosis of localized scleroderma was made and physiotherapy was started. No drug therapy was given. Five months after onset of disease severe scleroderma changes of the entire left upper extremity were even more

bling the dose produced the same effect and eventually a dose of 14 mEq/kg was required to maintain the plasma bicarbonate above the threshold the urine alkaline and to correct the acidosis (Fig. 2). The need for this very large dose was consistent with previous experience in treating proximal RTA (8).

In glycogen storage disease absence of the debranching enzyme leads to the accumulation in the liver of abnormal molecules of glycogen with a structure approaching that of the phosphorylase limit dextran (2). Histological studies of renal biopsy tissue from primary proximal RTA have been essentially normal (10) but in the isolated reports (3, 5, 9) of the appearances of the renal histology in cases of secondary RTA there is the suggestion that deposition of abnormal metabolites may cause disruption of the tubules in particular.

The occurrence together of two such uncommon disorders as Type III GSD and renal tubular acidosis is unlikely to be due to chance alone. The children were male and products of a consanguineous marriage and both conditions have an increased familial incidence. Indeed enzyme analysis of white cells from the five siblings of the first case demonstrated a brother who had a tenfold reduction in debranching enzyme activity consistent with Type III GSD.

The association could thus be explained by genetic linkage alone but equally it is possible to speculate that the accumulation of abnormal molecules of glycogen in the renal tubular cells was responsible for the observed defects of urinary acidification and this concept would be compatible with the findings of a relatively non-specific pattern of damage to tubular function.

It is important to actively exclude renal tubular lesions as a contributory cause for failure to thrive in all patients with GSD and if demonstrated such defects should be fully

characterised since both management and prognosis are materially affected.

ACKNOWLEDGEMENTS

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REFERENCES

- 1 Fernandes J & van de Kamer J H Hexose and protein tolerance tests in children with liver glycogenosis caused by a deficiency of the debranching enzyme system *Paediatrics* 41 935 1969
- 2 Garancis J C Panares R R Good T A & Kuzma J F Type III Glycogenosis: A biochemical and electron microscope study *Lab Invest* 27 468 1970
- 3 Harris R C & Olav C Liver and kidney glucose 6-phosphatase activity in children with normal and diseased organs *J Clin Invest* 46 336 1974
- 4 Lampert F Mayer H Tocci P M & Nyhan W L Fanconi syndrome in Glycogen Storage Disease In Nyhan (ed.) *Amino acid metabolism and genetic variation* McGraw Hill New York 1967 p 753
- 5 Morgan H G Stewart W K Lowe K G Stowers J M & Johnstone J H Wilson's disease and the Fanconi syndrome *Quart J Med* 31 361 1967
- 6 Sebastian A & Morris R C Renal Tubular Acidosis *Clin Nephrol* 7 216 1977
- 7 Rodney Howell R The Glycogen Storage Diseases In J B Stanbury J B Wyngaarden and D S Fredrickson (eds.) *The metabolic basis of inherited disease* McGraw Hill New York 1978 4th ed p 137
- 8 Seldin D W & Wilson J D Renal tubular acidosis In J B Stanbury J B Wyngaarden and D S Fredrickson (eds.) *The metabolic basis of inherited disease* McGraw Hill New York 1978 4th ed p 1618
- 9 Schoen E J Lowes Syndrome *Am J Med* 27 181 1959
- 10 Soriano J R Boichus H Stark H & Edelman C M Proximal renal tubular acidosis: A defect in bicarbonate reabsorption with normal urinary acidification *Pediatr Res* 1 81 1967

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We found no description in the literature of localized scleroderma in a patient with IgA deficiency. This finding could be a chance association since selective IgA deficiency could occur without disease status. However, since IgA deficiency has been found in higher than expected rates in patients with connective tissue diseases (2, 3), this might also be the case in localized scleroderma.

The onset of the disease following varicella is of interest. Viruses may play an important role in autoimmune diseases by adversely modifying the host immune response and promoting the formation of autoantibodies and auto-sensitized cells.

Moreover, in some autoimmune diseases virus particles were identified in affected tissues, as shown by Gyorkey et al. (5).

We suggest a possible relationship between the development of localized scleroderma in this girl, IgA deficiency, a virus infection (varicella) and anti-DNA antibodies. Although the findings mentioned above may be coincidental, the development of a severe connective tissue disease in only one limb, which was heavily involved by varicella, not only bears a serious prognosis for the involved limb, but

also presents a fascinating enigma which deserves further study.

REFERENCES

- 1 Cassidy J T & Burt A. Isolated IgA deficiency in juvenile rheumatoid arthritis. *Arthritis Rheum* 10: 27, 1967.
- 2 Cassidy J T, Friedlander R, Schmickel M, Petty R & Sullivan D. Impaired lymphocyte transformation in patients with connective tissue disease and selective immunoglobulin A (IgA) deficiency. *Arthritis Rheum* 12: 659, 1969.
- 3 Cassidy J T, Burt A, Petty R & Sullivan M. Selective IgA deficiency in connective tissue diseases. *N Engl J Med* 280: 275, 1969.
- 4 Chazen E M, Cook C D & Cohen Y. Focal scleroderma: report of 11 cases in children. *J Pediatr* 60: 385, 1967.
- 5 Gyorkey F, Sinkovics J G, Min A W & Gyorkey P. A morphologic study on the occurrence and distribution of structures resembling viral nucleocapsids in collagen diseases. *Am J Med* 53: 148, 1972.
- 6 Hanson V, Drexler E & Kornreich M. DNA antibodies in childhood scleroderma. *Arthritis Rheum* 13: 798, 1970.

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Fig. 1 Thinning of the epidermis, flattening of the rete ridges. Thickening of the dermis showing hypertrophy and sclerosis of the collagen bundles enclosing groups of sweat glands surrounded by inflammatory infiltrates.



Fig. 1b The muscle beneath the involved skin shows interstitial myositis.

prominent than on initial examination they included besides remarkable tightness of the skin vitiligo and shiny skin. One year after onset of disease there was no essential change in the clinical findings and atrophy of the left upper extremity was more pronounced. Left arm circumference 10 cm above the elbow was 2.5 cm smaller than right arm circumference at the same level. Left forearm circumference 6 cm below the elbow was 2 cm smaller than right forearm circumference at the same level. Left arm and forearm are each 1 cm shorter than their right counterparts. The girl cannot clench her left fist, she has no flexion in her left wrist and only 30° wrist extension; she lacks 35° of elbow flexion and elbow extension is limited by 35°. Abduction of left shoulder is limited by 15°. X-ray of the left upper extremity showed no calcification.

Since her initial visit in February 1976 sedimentation rate gradually decreased to 10/32 (Westergren), anti-nuclear factor and anti-DNA antibodies disappeared from serum. IgA deficiency was reconfirmed.

DISCUSSION

In their excellent review of nineteen cases of localized scleroderma in children, Chazen et

al (4) stressed the need for further studies in order to elucidate a possible role played by immunological processes in the etiology of this condition. Hanson et al (6) found antibodies to DNA in seven out of eighteen children with localized scleroderma while they found none in twenty-four children with dermatomyositis. During the acute stage of her disease which was accompanied by a high sedimentation rate and diffuse mononuclear infiltration of the involved extremity our patient also had positive antinuclear antibodies and antibodies to DNA but parallel to the return to normal of the sedimentation rate antinuclear antibodies disappeared.

Of special interest is the absence of IgA in the girl as well as in her father's serum and saliva. Cassidy et al (7) have described isolated IgA deficiency in juvenile rheumatoid arthritis (1) and other connective tissue diseases (2, 3).

NEW BOOKS RECEIVED

- D W Smith *Introduction to clinical pediatrics* (2/c)
Holt Saunders Ltd Eastbourne £7 00 ISBN 0-7116-8396-7
- G Sterky & L Mellander *Birth weight distribution—an indicator of social development* SAREC Report R 2 1978 95 pp SAREC/WHO Workshop 1978 Copies available on request at SAREC c/o SIDA 10525 Stockholm Sweden ISSN 0348 76 11
- R H Pierce M W Mainen & J F Bosma *The cranium of the newborn infant* An atlas of tomography and anatomical sections Superintendent of Documents U S Government Printing Office Washington D C 20407 DHEW Publ No (NIH) 78 788 148 pp \$6 50
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- R M Reece Reece Chamberlain *Manual of emergency pediatrics* (7/c) 771 pp Holt Saunders Ltd Eastbourne 1978 £10 75 ISBN 072167498-4
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- Major mental handicap methods and costs of prevention* Ciba Foundation Symposium 59 Elsevier Excerpta Medica/North Holland 1977 236 pp illus No price given ISBN 0-444-90033-0
- P G Ransley & M A Risdon *Reflux and renal scarring* British Journal of Radiology Supplement 14 1978 pp illus £3 60 (members of the British Institute of Radiology £3 00) ISBN 0306-8854
- A Altman & A Schwartz *Malignant diseases of infancy childhood and adolescence* In A J Schaffer & M Markowitz (eds.) *Major problems in clinical pediatrics* vol XVIII 531 pp illus W B Saunders Company Philadelphia London Toronto 1978 No price given ISBN 0-7716-1717 1

SOME CHARACTERISTICS OF TRANSCUTANEOUSLY MONITORED OXYGEN PARTIAL PRESSURE IN NORMAL NEWBORNS

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ABSTRACT Lofgren O and Jacobson L (Department of Obstetrics and Gynecology, General Hospital, University of Lund, Malmö, Sweden) Some characteristics of transcutaneously monitored oxygen partial pressure in normal newborns. *Acta Paediatr Scand* 68: 789-794, 1979.—The transcutaneous oxygen partial pressure (P_{tcO_2}) was monitored in 50 healthy, normally and spontaneously delivered newborns. Measurements were performed during the first to fourth day of life. The electrode temperature was 44.5°C . The mean P_{tc} level recorded during about 45 min was 9.2 kPa (SD 1.4) recorded from the minute-to-minute values. The P_{tc} level normally oscillated to a certain extent and the oscillations were closely related to the breathing pattern of the patient. When the patient fell asleep during measurement, the normal oscillating pattern was replaced by a silent pattern. During crying the P_{tc} level showed four main reaction patterns. A decrease in the P_{tc} level could be observed during breast feeding. One child recently fed vomited a small amount of breast milk after a short period of crying and apparently had a laryngospasm shown by a sudden drop in the P_{tc} level without any other signs of discomfort. The study shows that P_{tc} (and thus also P_a) very sensitively reacts to changes in activity. This implies that earlier used methods for determination of P_a might give values that are not representative for the steady state as the sampling method per se might influence the recorded P_{tc} value.

KEY WORDS Transcutaneous monitoring, P_{tc} monitoring, normal P_{tcO_2} levels, P_{tc} and state of activity.

Transcutaneous oxygen partial pressure (P_{tc}) is not equivalent to arterial P_{aO_2} (P_{aO_2}) rather P_{tcO_2} is a new parameter with its own characteristics. However, P_{tc} has a very consistent relationship to P_{aO_2} in each patient and P_{tcO_2} very reliably reflects even small changes in the central oxygenation given a constant and adequate skin circulation at the measurement site (20).

Systematic studies of the P_{tc} monitoring method thus far reported have been primarily concerned with the reliability of P_{tc} measured in terms of its correlation with P_{aO_2} in intermittently obtained arterial blood samples (5, 6, 7, 9, 10, 12, 14, 19, 20, 24, 25, 26, 27). Most of these studies report on high correlations for diseased newborns. In some studies, however, correlations are

reported for normal patients breathing air (6, 10) and breathing oxygen (10). In 1976 Huch et al. (10) reported P_{tcO_2} to be 8.7 kPa in less than 5% of normal newborns (electrode temperature 45°C and 44°C for normal and preterm infants, respectively).

The following study was performed in order to study the range of P_{tcO_2} in normal newborns breathing air during a specified monitoring situation and to study changes in the P_{tcO_2} level during different states of activity.

EQUIPMENT AND PROCEDURE

The equipment used was a Radiometer TCM 1 unit (Radiometer, Denmark). A $2.5\text{ }\mu\text{m}$ teflon membrane was used. The electrode temperature was set at 44.5°C (anode temperature, the skin surface temperature was assumed to be 43°C). Immediately before and after every recording the electrode was calibrated and recalibrated in an

BOOK REVIEWS

A Peters *Bewegungsanalysen und Bewegungstherapie im Säuglings und Kleinkinderalter* Gustav Fischer Verlag Stuttgart New York 1977 138 pp illus DM 26 00 ISBN 3-437 00228 7

The author is physiotherapist in Heidelberg and the book is intended to be of help in the early treatment of infants and pre school children with motor handicaps or late motor development. The book is in German. It is easy to read and gives a very good description of normal motor development in the first year. It also provides several examples of simple ways of treating a child in order to facilitate a normal sensory motor development. The figures are very clear and instructive with the child pictured in different positions with arrows marking how the therapist can give support to the child and initiate movement. The method of therapy described in the book is greatly influenced by the Bobath method and partly by the Voita method.

This book can be recommended to doctors and therapists dealing with small motor handicapped children and will be especially useful for physiotherapists treating these children.

Kerstin Odlund

A Altman & A Schwartz *Malignant diseases of infancy, childhood and adolescence* In A J Schaffer & M Markowitz (eds.) Major Problems in Clinical Pediatrics vol XVIII 531 pp illus W B Saunders Company Philadelphia London Toronto 1978 No price given ISBN 0-7216-1212 1

It is a difficult task to write on malignant diseases in children when the primary recipient of the book is meant to be the practising paediatrician rather than specialist in the field. The authors of the present volume judiciously place much emphasis on diagnosis and the natural history of malignancy as well as on complications of its treatment.

The first seven chapters deal with general aspects of cancer in childhood and the chapters on diagnosis and oncologic emergencies are especially valuable. The chapter on radiotherapy is concise and informative while that on chemotherapy is overloaded with details. The next fifteen chapters describe individual malignancies in a comprehensive and didactic way. Some of them such as the chapters on tumours of germ cell origin or on tumours of the sexual organs are doubtless too extensive for the needs of a general paediatrician but may serve as a valuable reference for those specially interested in the field.

Figures, tables and classification or diagnostic schemes are of high quality throughout the book. References are up to date but sometimes unnecessarily numerous.

Some minor remarks may be pertinent in case of a 2nd edition of the book.

p 200 — description of consolidation therapy in leukemia may be misleading — it does include a short course of chemotherapy to further reduce the leukemic population but not prophylactic treatment of sanctuary areas.

p 335 — stage IV neuroblastoma is described in the text but not exactly defined together with other stages.

p 365 — urine should be collected for catecholamine determination in all cases where neuroblastoma is a possible differential diagnosis not only if neuroblastoma is suspected.

p 434 — histologic grading of immature teratomas is given both on this page and on p 448. On the other hand no staging system is presented for ovarian neoplasms though treatment and its results are related to stage.

On the whole the book can be recommended not only for the general paediatrician for whom it was primarily written but also for the oncologic paediatrician and other members of the pediatric oncology team.

Stanislav Garovic

Major mental handicap: methods and costs of prevention Ciba Foundation Symposium 59 Elsevier Excerpta Medica/North Holland 1977 236 pp illus No price given ISBN 0-444 90033 1

The book covers a symposium on the cost of preventing major mental handicap held in London November 1977. Most of the participants came from England and hence several of the reported studies related to that country. Nevertheless the studies and discussions are relevant to the situation in most western countries.

In the symposium major mental handicaps were discussed in relation to prenatal, perinatal and postnatal events. Several well known investigators from different countries presented their views and own experience on each of these subjects. Besides this the possibility of risk assessment during pregnancy and in the perinatal period was discussed. Of special interest are the first chapters dealing with the prenatal aspects and stressing the importance of good antenatal care and centralization of delivery and neonatal services. The chapters on neonatal intensive care deal mainly with mortality rates but some information on reduced handicap rates is also given.

At the end of each presentation there is a discussion that critically analyses what has been presented. The last two chapters deal with the application of cost benefit analysis to the prevention of mental handicap both from a theoretical and a practical point of view.

This book raises and discusses several important questions. Everyone working in the field of perinatology, obstetrics and paediatrics should be interested in reading it.

Margareta Eriksson

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Transcutaneous oxygen partial pressure (P_{tc}) is not equivalent to arterial P_{aO_2} rather P_{tcO_2} is a new parameter with its own characteristics. However, P_{tc} has a very consistent relationship to P_{aO_2} in each patient and P_{tcO_2} very reliably reflects even small changes in the central oxygenation given a constant and adequate skin circulation at the measurement site (10).

Systematic studies of the P_{tcO_2} monitoring method thus far reported have been primarily concerned with the reliability of P_{tc} measured in terms of its correlation with P_{aO_2} in intermittently obtained arterial blood samples (4, 7, 9, 10, 12, 14, 19, 20, 24, 25, 26, 27). Most of these studies report on high correlations for diseased newborns. In some studies, however, correlations are

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The following study was performed in order to study the range of P_{tcO_2} in normal newborns breathing air during a specified monitoring situation and to study changes in the P_{tcO_2} level during different states of activity.

EQUIPMENT AND PROCEDURE

The equipment used as a Radiometer TCM 1 unit (Radiometer, Denmark). A 5 µm teflon membrane was used. The electrode temperature was set at 44.5°C (anode temperature, the skin surface temperature was assumed to be 43.5°C). Immediately before and after every recording the electrode was calibrated and recalibrated in an

Table 1 *Ptc*₀₂ in normal newborns

N	Electrode temp (°C)	Electrode drift (kPa)	Mean F_{IC} (kPa)	Mean birth weight (g)	Mean recording time (min)	Mean measuring time (min)
50	44.5	-0.2 (S.D. 0.8)	9.2 (S.D. 1.4)	3,579 (S.D. 448.0)	44.5 (S.D. 11.70)	60.0 (S.D. 6.11)

antiseptic water solution of Urolicide[®] (American Latex Corporation Indiana USA) bubbled through with air. The temperature of the calibration solution was 43.5°C.

The infants usually recently fed and changed were supine on a nursing table and partly covered by blankets. The room temperature was approximately 25°C. A newly calibrated electrode was applied by means of a double adhesive tape in the subclavicular area. After stabilization of the P_{tc} level recording was performed for about 45 min. During the recording time the spontaneous fluctuations of the P_{tc} levels were studied as was the average basal level. At the end of the recording some of the infants were made to cry by tapping the sole of the foot. In two cases the infant was given to the mother for breast feeding at the end of the recording with the electrode still in place. After the recording the electrode was recalibrated.

PATIENTS

Recordings were performed in 50 healthy normally and spontaneously delivered newborns with a mean birth weight of 3579 g (Table 1). The measurements were performed during the first to fourth day of life (Fig. 1).

RESULTS

Electrode drift

The mean electrode drift obtained as the difference in calibration values before and after

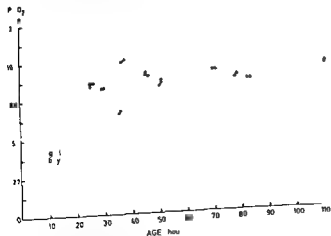


Fig. 1 Material Age at start of measurement and mean P_{TC} level calculated from the minute to minute values

the recording was -0.2 kPa (S.D. 0.8) showing a small mean declining drift (Table 1)

Pico,

All recordings showed a more or less wave like pattern. It was apparent that although the pattern was dependent on the activity of the infant it could also differ among infants in the same state of agitation. Thus when the infant was alert or irritated the oscillations were frequent with comparatively high amplitudes. However when the newborn was asleep smooth pattern (silent pattern) appeared. An example of this is shown in Fig 2 with the infant alert but not crying during the first part of the recording and asleep during the latter part.

The maximal range within which the P_{tCO_2} level oscillated in the same patient was 6.4 kPa and the minimal range of oscillations recorded in one patient was 0.6 kPa. The range of P_{tCO_2} most commonly recorded among patients was about 2.7 kPa.

and from these values a mean P_{tCO_2} level was obtained for the patients. The mean P_{tCO_2} level was obtained for the patients. The mean P_{tCO_2} level recorded was for the total material 9.2 kPa (S.D. 1.4) (Table 1).

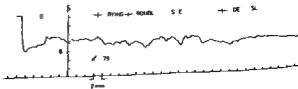


Fig. 2 The effect of different stages of activity on Ptc_0 level

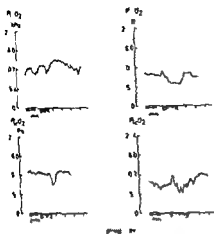


Fig 3 Four common reaction patterns of the $P_{tc}O_2$ level during crying

Crying was produced in 30 of the 50 infants by tapping the sole of the foot for about 2 min. During the crying period the $P_{tc}O_2$ level showed varying reaction patterns. The four most common patterns are shown in Fig 3. In most infants after an initial brief increase the $P_{tc}O_2$ level started to decrease (Fig 3 1 2 4). The mean decrease in the $P_{tc}O_2$ level during crying was 2.2 kPa (SD 1.0). Eleven of the infants became calm again immediately after two min crying; the $P_{tc}O_2$ levels in these children returned to their original levels on the average 75 sec after crying had stopped (Fig 3 2). In five of these 11 infants the $P_{tc}O_2$ level continued to fall even though the child was calm. In these latter five infants the $P_{tc}O_2$ level reached a mean maximal decrease of 3.4 kPa on the average 34 sec after they had stopped cry

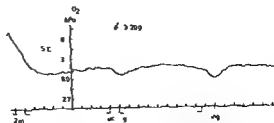


Fig 5 Reaction of the $P_{tc}O_2$ level to breast feeding

ing (Fig 3 3). The original $P_{tc}O_2$ level was reached after a mean of 104 sec. In two children the $P_{tc}O_2$ level increased immediately when they started to cry, as in most cases but then fell only a few kPa below the original level during the 2 min long crying period (Fig 3 1).

During one recording shown in Fig 4 the recently fed infant vomited a small amount of breast milk after a short crying period. After this the child apparently had a laryngospasm shown by a sudden drop in the $P_{tc}O_2$ level recorded. The $P_{tc}O_2$ level decreased to a minimal level of 3.3 kPa. The child was lying on its back almost asleep during the incident. No cyanosis or obvious signs of discomfort could be observed by the experimenter. The child was observed intensively without any intervention by the experimenter; after a few sec the $P_{tc}O_2$ level increased to its original and normal level and remained there for the rest of the recording. The subsequent course was uneventful.

At the end of the recordings two infants were breast fed by their mothers with the electrodes still in place (Fig 5). A decrease in the $P_{tc}O_2$ level could be observed during sucking in these newborns as in those given a dummy teat for consolation when crying during the first part of the recording. When the baby intermittently stopped sucking the $P_{tc}O_2$ level immediately rose to its original level.

DISCUSSION

$P_{tc}O_2$ should be regarded as a new parameter with its own characteristics such as

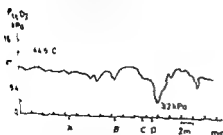


Fig 4 Reaction of the $P_{tc}O_2$ level to a brief laryngospasm after vomiting (D). (A) Troubled sleep (B) crying (C) stop of crying (D) vomiting

normal range and normal value. A normal range is by definition the range between two extreme values observed in a defined population by a well defined laboratory procedure. For Ptc_{O_2} measurements the laboratory procedure is not well defined and some studies do not even report on the laboratory procedure used.

Fenner et al (6) and Huch et al (10) have reported mean Ptc_{O_2} levels of 12.0 kPa and 11.5 kPa respectively in newborns. In both studies Ptc_{O_2} was reported as intermittent values related to P_{aO_2} in arterial samples obtained by puncture of the radial artery. The skin temperature was 43.0°C in both studies.

Huch et al (13) reported a normal range of arterial P_{O_2} in non-crying neonates from about 8.7 kPa to 13.4 kPa. In only 5% the Ptc_{O_2} level was said to be less than 8.7 kPa in non-crying neonates. The electrode temperature used for fullterm infants was 45°C and for premature infants 44°C. The equipment used was the original Huch instrument with three cathodes.

The discrepancy between the mean Ptc_{O_2} level obtained in the current study and the mean level reported by others could to some extent be accounted for by differences in the calibration procedure and differences in electrode temperature. It must be stressed as mandatory to report on both the calibration temperature and on the electrode temperature used during measurement in reports of Ptc_{O_2} . For the current instrument the electrode temperature 44.5°C has been shown to produce a high correlation between P_{aO_2} and Ptc_{O_2} (20) and in measurements with two simultaneously operating electrodes the electrode temperature (anode temperature) of 44.5°C has been shown to produce an optimal arterialization (21).

The mean Ptc_{O_2} level obtained in the current study concurs with the level reported for P_{aO_2} obtained intermittently from umbilical artery catheters in healthy newborns (16, 17).

As was shown in the current study crying usually started with a short period of tachy-

pnea and hyperventilation. This produced an initial increase of the Ptc_{O_2} level followed by a decrease. Thus puncture of the radial artery per se affects both the P_{aO_2} and the Ptc_{O_2} level so that the P_{aO_2} recorded by both methods would not reliably reflect P_{aO_2} in the steady state. Other studies with the current instrument and the current laboratory procedure (19, 20) have shown a high correlation between Ptc_{O_2} and arterial samples obtained from umbilical artery catheters with only a very small difference between P_{aO_2} and Ptc_{O_2} during normoxemia.

The present study showed that the pattern of oscillation was dependent on the breathing pattern of the infant and thus on the state of activity. When the child was alert or irritated the Ptc_{O_2} level showed vigorous oscillations. These oscillations were smoothed out and changed for a silent pattern if the child was asleep. The mean level of Ptc_{O_2} seemed to be the same during both states of activity however intermittent apnea i.e. during breast feeding and sucking could result in rather deep and broad dips of the Ptc_{O_2} level. Crying almost always resulted in a decrease of the Ptc_{O_2} level after 5–10 sec. This has also been found by others (8, 10, 11, 14). However the Ptc_{O_2} response to crying does not seem to be uniform showing four main patterns in the present study. Usually the Ptc_{O_2} level decreased during crying and returned to a normal level when the child stopped crying. In some infants the decrease of the Ptc_{O_2} level continued after the crying had stopped. Previous studies of the physiology of crying patterns in children have mostly focused on respiratory volumes (18). The Ptc_{O_2} monitoring method thus offers a new possibility for studying the physiology of crying as certain crying techniques increase the Ptc_{O_2} level whereas others cause a decrease in the Ptc_{O_2} level.

During recording one infant had a laryngo spasm due to vomiting. The immediate decrease in the recorded Ptc_{O_2} level is evidence of the sensitivity of the method. The child's Ptc_{O_2} level declined to a very low level for a

short time although there was no sign of discomfort. The condition was spontaneously relieved. Incidents of this kind are probably very common in newborns. If however the P_{O_2} level of the infant were initially low, the brief apnea of this type could prove harmful, beginning a vicious circle (2, 3 for a review see 15). A laryngeal chemosensitivity producing apnea has even been proposed as one of several possible mechanisms accounting for sudden infant death (1, 4).

The electrode drift calculated as the difference in calibration value before and after the recording was low. Lofgren (22, 23) has shown that the electrode drift calculated in this way is unsatisfactory as a standard for calculation of the electrode drift during measurement. That finding also corresponds to ideas suggested by James (14). The P_{tCO_2} oscillatory pattern—and perhaps also the stabilization pattern—seem to be better indices of the reliability of the P_{tCO_2} recording than is the recalibration value (19). It has been suggested (19) that a silent pattern found in a spontaneously breathing patient who is not sleeping, might indicate that the electrode is applied in a non optimal measurement site giving a less reliable recording.

REFERENCES

- Barker T L & McGinty D J. Reversal of cardiopulmonary failure during active sleep in hypoxic kitten. Implications for sudden infant death. *Science* 195 419 1977.
- Brady J P & Cerulli E. Chemosensitive reflexes in the newborn infant. Effects of varying degrees of hypoxia on heart rate and ventilation in a warm environment. *J Pediatr* 119 631 1966.
- Cross H W & Oppé T E. The effect of inhalation of high and low concentrations of oxygen on the respiration of the premature infant. *J Physiol* 117 14 1955.
- Downing S L & Lee J C. Laryngeal chemosensitivity: A possible mechanism for sudden infant death. *Pediatrics* 55 640 1974.
- Eberhard P, Meind W, Jans F & Hamacher K. Oxygen monitoring of newborns by am electrodes. Correlation between arterial and cutaneously determined PO_2 . In E. Briley & R. E. Baer (eds), *Oxygen Transport in the Neonate*. Plenum Press, New York 1971.
- Fenner A, Müller H, Busse H G, Junge M & Wollsdorff J. Transcutaneous determination of arterial oxygen tension. *Pediatrics* 55 274 1975.
- Fris Hansen H, Marstrand Christiansen P & Vesterager P. Transcutaneous measurement of arterial blood oxygen tension with a new electrode. In L. Stern, B. Fris Hansen & P. Kildeberg (eds), *Intensive Care in the Newborn*. Masson Publishing Inc, New York 1976 p 759.
- Haschke F, Hackel F & Hohenauer L. Erfahrungen mit der transkutanen (unblutigen) PO_2 Registrierung beim Atemgestörten Neugeborenen. *Klin Padiatr* 188 73 1976.
- Huch A, Huch R & Lubbers D W. Quantitative polarographische Sauerstoffdruckmessung auf der Kopfhaut des Neugeborenen. *Arch Gynaekol* 207 443 1969.
- Huch R, Huch A, Meinzer K & Lubbers D W. Eine beheizte P_{tO_2} oberflächen elektrode zur kontinuierlichen Überwachung des PO_2 beim Menschen. Vortrag Medizin Technik Stuttgart 1972.
- Huch R, Huch A & Lubbers D W. Transcutaneous measurement of blood PO_2 (tPO_2)—Method and application in perinatal medicine. *J Perinat Med* 1 183 1973.
- Huch R, Lubbers D W & Huch A. Reliability of transcutaneous monitoring of arterial PO_2 in newborn infants. *Arch Dis Child* 49 213 1974.
- Huch R, Huch A & Lubbers D W. Die kontinuierliche Kontrolle des arteriellen PO_2 und ihre Bedeutung für die Überwachung von Mutter und Kind. *Biomed Tech* 19 87 1974.
- James L S. Development and clinical evaluation of blood gas sensors for continuous monitoring (adult and neonate). *US Dept of Commerce National Technical Information Service* 1 1976.
- Kattwinkel J. Neonatal apnea. Pathogenesis and therapy. *J Pediatr* 90 347 1977.
- Koch G & Wendel H. Comparison of pH, carbon dioxide tension, standard bicarbonate and oxygen tension in capillary blood and in arterial blood during the neonatal period. *Acta Paediatr Scand* 56 10 1967.
- Koch G & Wendel H. Adjustment of arterial blood gases and acid base balance in the normal newborn infant during the first week of life. *Biol Neonat* 12 136 1968.
- Long E C & Hull W E. Respiratory volume flow in the crying newborn infant. *Pediatrics* 7 373 1961.
- Lofgren O. On transcutaneous PO_2 measurements in humans. Some methodological, physiological and clinical studies. Thesis University of Lund 1978.
- Lofgren O, Hennrikson P, Jacobson L & Johansson O. Transcutaneous PO_2 monitoring in neonatal intensive care. *Acta Paediatr Scand* 67 693 1978.
- Lofgren O & Jacobson L. The influence of different electrode temperatures on the recorded transcutaneous PO_2 level. Accepted for publication. *Pediatrics*.
- Lofgren O. The recalibration value after P_{tO_2} measurement. Submitted for publication.

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TRANSEPIDERMAL WATER LOSS IN NEWBORN INFANTS

III Relation to Gestational Age

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ABSTRACT Hammarlund K. and Sedin G (Department of Paediatrics University Hospital Uppsala Sweden) Transepidermal water loss in newborn infants. III. Relation to gestational age. *Acta Paediatr Scand* 68 795 1979. — Using a method described earlier the evaporation rate (ER) was studied in different humidities in 12 newborn infants born after 25 to 30 weeks of gestation and 10 infants born after 32 to 35 weeks. Transepidermal water loss (TEWL) was estimated in 32 infants born after 25 to 39 weeks of gestation. The ER values were highest in the infants with the lowest gestational age and the susceptibility to changes in ambient humidity was also greater at lower gestational ages. An exponential relationship was found between TEWL and gestational age. TEWL being 15 times higher in infants born after 33 weeks of gestation than in full term infants.

KEY WORDS Water loss, water balance, newborn infants, neonatal intensive care, gestational age.

Water, electrolytes and calories are frequently supplied by the parenteral route in newborn infants when oral feeding is difficult for some reason, e.g. prematurity, asphyxia or pulmonary disease. The amount of fluid to be given is usually calculated in relation to body weight or body surface area, with adjustments according to postnatal age.

Disturbances of water balance in the neonatal period are not due solely to the supply of fluid to the infant. Other factors to be considered are water losses to the environment and changes in the body distribution of water (4).

Hitherto no accurate clinical and laboratory methods for routine checking of the state of hydration and of insensible water loss (IWL) have been available. IWL, the major part of which is cutaneous (9), has mainly been investigated in full term infants or infants with a low birth weight, while no thorough study of this loss has been made in relation to gestational age and environmental factors.

In our earlier investigations of the evaporation rate (ER) from the skin of full term

infants a clear relationship between ER and ambient humidity was found and a method for determination of transepidermal water loss (TEWL) was proposed (5, 15). Changes in TEWL in relation to activity and body temperature have been found (6, 7, 16). A very high TEWL in preterm infants has been noted and reported in preliminary communications (7, 16).

The purpose of this investigation was to study the evaporation rate from the skin of newborn preterm infants during the first day of life and to estimate the TEWL in relation to gestational age.

SUBJECTS

ER was measured at different ambient humidities on 19 infants born after 25 to 30 weeks of gestation and on 10 infants born after 32 to 35 weeks of gestation (Table 1, measurement series 1a and 1b). Further measurements of ER were performed and TEWL was estimated (3) in 3 infants born after 25 to 39 weeks of gestation (Table 1, measurement series 2). All infants were appropriate for gestational age (AGA) (10). The gestational age was estimated from pregnancy data and as described by Finnström (3) and also in most cases with the en-

- 23 Lofgren O. Drift of the transcutaneous oxygen monitoring electrode during in vivo measurement. Submitted for publication.
- 24 Swinström S, Villi Elisabet I, Cardona I, Cardenas A, Méndez Bauer C & Rooth G. Transcutaneous PO₂ measurements in seriously ill newborn infants. *Arch Dis Child* 50: 913, 1975.
- 25 Swinström S & Rooth G. The value of transcutaneous PO₂ measurements in a newborn infant in shock. *Pediatrics* 59: 304, 1976.
- 26 Vermold H, Hinne V, Hopner F & Riegel K. P. Kritische Anmerkungen zur transkutanen PO₂ Messungen bei Risikoneugeborenen. *Monatsschr Kinderheilkd* 123: 459, 1975.
- 27 Vermold H, Onken H, Hopner F & Riegel K. Transcutaneous monitoring of PO₂ in the sick newborn. In L. Stern, B. Frus Hansen & P. Kildeberg (eds), *Intensive Care in the Newborn*. Masson Publishing USA 1976, p. 269.

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Table 2 Skin temperature (T_{kin}) and ambient temperature (T_{mb}) in series 1 and 2

In- fant	Series 1		Series 2	
	$T_{\text{amb}} \pm \text{S.D.}$ (°C)	$T_{\text{mb}} \pm \text{S.D.}$ (°C)	\bar{T}_{kin} (°C)	\bar{T}_{mb} (°C)
1	36.7±0.1	39.3±0.5	36.1	38.3
2	35.9±0.1	39.8±0.7	36.6	38.8
3	36.0±0.2	39.6±0.7	35.9	39.9
4	36.4±0.1	38.6±0.9	36.0	39.0
5	36.5±0.1	38.8±0.5	36.5	39.0
6	36.6±0.1	39.5±0.4	36.3	38.9
7	36.4±0.3	36.5±0.6	36.5	39.8
8	35.7±0.3	36.7±0.7	36.6	36.9
9	36.7±0.3	37.3±1.0	35.8	36.4
10	36.0±0.1	35.3±0.8	36.5	36.6
11	36.3±0.0	37.5±0.4	36.4	34.0
12	36.0±0.1	36.6±0.6	36.5	37.8
13	36.9±0.0	36.1±0.5	35.8	36.5
14	36.1±0.1	35.3±0.5	36.1	35.5
15	36.3±0.1	34.6±0.7	36.1	37.6
16	35.6±0.1	34.8±0.4	36.1	34.6
17	36.1±0.1	34.4±0.6	36.0	34.0
18	35.5±0.0	33.7±0.7	35.3	33.4
19	34.9±0.3	34.7±0.9		
20	35.9±0.1	34.5±0.4	36.1	34.7
21	35.9±0.1	34.8±0.4	35.9	34.9
22			36.7	33.8
23	35.6±0.1	35.7±0.7	35.8	35.1
24			35.1	33.4
25			35.5	35.0
26			36.2	34.0
27			36.3	35.8
28			35.7	33.2
29			35.6	33.5
30			35.4	33.8
31			35.0	33.8
32			34.8	33.7
33			36.0	34.4

on the temperature used in the period before the measurements

1 Measurement of ER at different ambient humidities

With the infant in the prone position intermittent measurements of ER were made on an interscapular skin surface while RH_{mb} was increased in steps from 70% to 60% or decreased from 60% to 70% through a change in the relation between dried and humidified air in the incubator at a constant air flow. After each change in RH_{mb} steady state was reached in 5–10 min.

Estimation of transepidermal water loss

With the infant in the lateral position and with RH_{amb} maintained at 50% ER was measured on the chest (a) on an interscapular skin area (b) and on a buttock (c). The transepidermal water loss (TEWL, g/m²h) i.e. the cutaneous water loss per unit area was calculated as described previously (5) using the equation

$$\text{TEWL} = 0.9 \bar{ER}_t \bar{T}_{\text{kin}} + 1.37 \quad (1)$$

Skin temperatures were recorded from the areas where the ER measurements were made

TREATMENT OF DATA

Data were obtained for ER, RH_{amb}, P_{aO_2} , T_{mb} , T_{kin} and T_{core}

The arithmetic mean of the three measured skin temperatures for each TEWL was designated \bar{T}_{kin} (Table 2). Regression analysis was performed by a computer.

RESULTS

1a Evaporation rate at different ambient humidities in infants born after 32 to 35 weeks of gestation compared with that in full term infants

When ER was measured on an interscapular skin area in the 10 infants born after 32 to 35 weeks of gestation higher values were obtained at a low RH_{mb} than at a high one (Fig. 1 upper curve). This is consistent with the findings in fullterm infants (Fig. 1 lower curve) made in a previous study (5). The values obtained in these preterm infants were higher than those in full term infants at corresponding RH_{amb}. For example at an RH_{amb} of 50% ER was 6.2 g/m²h compared with 3.5 g/m²h in the full term infants.

It was possible to keep T_{core} fairly constant in all but three of the infants with a gestational age of less than 35 completed weeks: the system for servo-control of skin temperature (AGA Medical Lidingö Sweden) supplied with the incubator was used.

To keep T_{core} between 36.0 and 37.0°C the servo-control was set at approximately 36.0°C and the regulating thermistor was attached to the skin of the abdomen. The servo-control adjusted the temperature in the incubator to a level high enough to maintain the abdominal skin temperature at the set level. As a safety circuit interrupts the supply of heat when the temperature of air circulating in the incubator reaches about 40°C some infants with a low gestational age did not reach an abdominal skin temperature of 36.5°C. The highest T_{core} was thus registered for infants of the shortest gestational ages (Tables 1 and 3) both in series 1 and in series 2.

When the servo-control was not used (infants of more than 34 completed weeks of gestation) the temperature in the incubator was set at 33.0–34.0°C depending on

Table 1 Infant data in series 1 and 2

n = completed weeks of gestation V = vaginal delivery CS = Caesarean section

Infant	Gestational age (w)	Delivery	Weight at birth (kg)	Length at birth (m)	Sex	Measurement series
1	25	V	0.825	0.350	F	1b ?
2	25	V	0.830	0.330	F	?
3	26	V	0.950	0.350	M	1b ?
4	26	CS	0.650	0.330	M	1b ?
5	27	V	1.070	0.360	M	1b ?
6	28	V	1.070	0.355	M	1b ?
7	28	CS	0.890	0.350	M	1b ?
8	29	V	1.600	0.410	F	1b ?
9	29	V	1.570	0.410	M	1b ?
10	30	CS	1.370	0.400	F	1b 2
11	30	V	1.420	0.405	F	1b ?
12	30	V	1.500	0.400	M	1b ?
13	30	CS	1.330	0.360	M	1b 2
14	32	CS	1.590	0.435	M	1a 1b ?
15	32	CS	1.670	0.405	M	1a 1b ?
16	33	V	2.150	0.430	F	1a 1b ?
17	33	CS	2.000	0.430	F	1a 1b ?
18	33	CS	2.090	0.450	M	1a 1b ?
19	33	V	1.900	0.440	M	1a 1b ?
20	34	CS	2.160	0.450	F	1a 1b
21	34	CS	2.250	0.460	M	1a 1b ?
22	34	V	2.000	0.450	F	1a 1b 2
23	34	V	2.130	0.455	M	?
24	35	V	2.320	0.450	M	1a 1b ?
25	38	V	3.070	0.470	F	?
26	38	CS	1.730	0.570	M	?
27	38	CS	1.620	0.525	M	?
28	38	CS	1.990	0.510	M	?
29	38	CS	1.750	0.500	M	?
30	38	CS	2.910	0.490	M	?
31	38	CS	3.770	0.500	M	?
32	39	V	3.650	0.530	M	?
33	39	V	2.850	0.500	F	?
34	39	CS	2.960	0.495	F	?

terin proposed by Dubowitz et al (1). The ER measurements were carried out during the first 24 h after delivery. The mean age at the start of the measurements was 10.7 h in series 1 and 8.1 h in series 2. The duration of the measurements was 18 h in series 1 and about 10 min in series 2. The infants were often asleep when the measurements were being made. When awake they were quiet and calm and showed little spontaneous motor activity (cf. 6). The respiratory and heart rates were normal for age.

METHODS

ER was measured by a method based on determination of the vapour pressure gradient in the air layer close to the skin surface (12-13, 14). This method which has been used earlier in studies on water loss in term infants (5, 6) allows accurate measurements and does not interfere with nursing routines. The equipment for measurement of ER also gives data on the ambient relative humidity (RH_{at}) and ambient vapour pressure

($P_{H_2O_{at}}$). The ambient air temperature (T_{at}), skin temperature (T_{sk}) and body temperature (T_{bt}) were measured with a YSI telethermometer (43TA and 400⁺ using probes 405, 421, 427 and 402 Yellow Springs, Ohio, USA). Recordings were made with a Watanabe recording system (Watanabe Instruments Corp., Tokyo, Japan).

MEASUREMENT PROCEDURE

All measurements were made with the naked infant placed in an incubator (AGA Mk41 or Mk241, AGA Medical, Lidingö, Sweden). The inflow of air to the incubator was kept at a constant level during the measurements in each infant and ranged between 8-15 l/min (cf. 5). The ambient humidity was regulated as described previously (5). T_{at} was registered from the skin areas where ER measurements were made. T_{bt} was recorded as deep rectal temperature and T_{mb} was measured in the central part of the incubator. By regulating T_{mb}

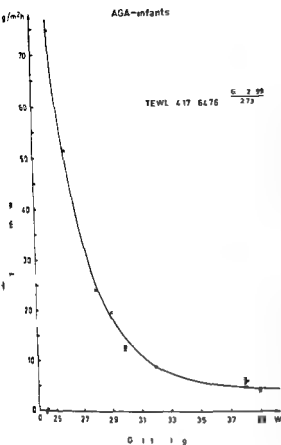


Fig 3 Transepidermal water loss (TEWL) in relation to gestational age (GA) AGA=appropriate for gestational age w=completed weeks of gestation

of life in newborn infants of different gestational ages. There are large dissimilarities in the state of hydration (4) in the control of body water (11) and in the ability to compensate for disturbances in water balance (for review see 8). Further little is known about the magnitudes of the water losses to the environment and the factors that influence them. This has resulted in wide divergences of opinion as to the amount of fluid to be supplied.

The investigation of insensible water loss (11) by Fantruff et al (2) showed that this was very high in infants with a birth weight less than 1250 g. No report appears to have been published concerning the relation between water losses and gestational age. In

order to separate the effects of intrauterine nutrition and degree of maturity only the latter has been dealt with in this study. Thus only preterm infants appropriate for gestational age (AGA) were included.

The present results elucidate the great differences in transepidermal water loss between preterm and full term infants and demonstrate an exponential relationship between gestational age and TEWL. This may explain the clinical observation that infants with very low birth weights without noticeable extreme water losses sometimes succumb to hypertonic dehydration (2) although the ordinary amount of fluid per surface area is supplied. Further the high transepidermal water loss may be one of the reasons for the more frequent occurrence of intracranial haemorrhage in preterm infants as this seems to be related to hyperosmolality (18).

In order to avoid hyperosmolality the use of larger amounts of fluid has been proposed but problems of overhydration are then frequent and patent ductus arteriosus may occur during the first week of life in the smallest infants (17).

It is clear from the exponential shape of TEWL gestational age curve (Fig 3) that differences in gestational age of 2-3 weeks might cause great changes in the transepidermal water loss which renders proper adjustments of the fluid supply to the infant difficult.

From Tables 1 and 2 it is clear that a much higher ambient air temperature was necessary to keep the body temperature between 36.0 and 37.0°C in the infants born after a short gestational period. As the ambient relative humidity was kept constant at 50% the vapour pressure in the ambient air ($P_{H_2O, amb}$) was higher in these measurements. The exponential curve would thus have been even steeper if the vapour pressure had been kept constant instead.

The results of this study also explain the tendency for infants of very low gestational age to lose body heat despite a high ambient temperature. The heat losses through eva-

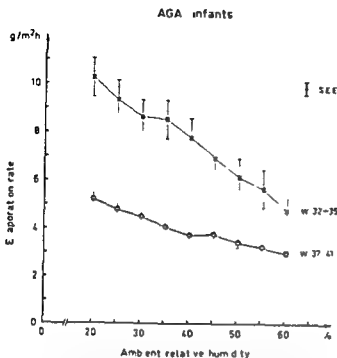


Fig. 1 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) in preterm and full term infants. S.E.E. = standard error of the estimate. AGA = appropriate for gestational age; w = completed weeks of gestation.

1b Evaporation rate at different ambient humidities in relation to gestational age

In Fig. 2 the relation between ER at different RH_{amb} and gestational age is shown. The figure includes data from 12 infants born after 25 to 30 weeks of gestation and 10 infants born after 32 to 35 weeks. The ER at an RH_{amb} of 50% was 44 g/m²h for the infant born after 25 weeks, 13.2 g/m²h for the infants born after 30 weeks and 6.0 g/m²h for the infants born after 34 weeks. The susceptibility to changes in ambient humidity as represented by the slope of each curve was greater at lower gestational ages.

2 Transepidermal water loss in relation to gestational age

In the 32 infants of gestational ages of 25 to 39 weeks in whom estimations of TEWL (g/m²h) were made, this loss showed an in-

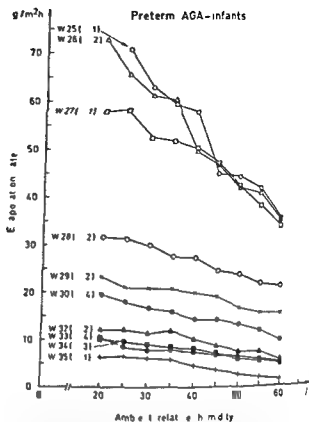


Fig. 2 The relation between evaporation rate (ER) and ambient relative humidity (RH_{amb}) in preterm newborn infants in different gestational age groups. AGA = appropriate for gestational age; w = completed weeks of gestation.

verse relationship to gestational age (Fig. 3). The relationship can be described by the following equation obtained by regression analysis:

$$TEWL = 4.17 + 64.76 e^{-\frac{(GA - 4.99)}{73}} \quad (2)$$

where GA is gestational age (weeks). The residual standard deviation of the equation is 2.72 ($n=32$, 28 degrees of freedom).

In infants with a gestational age of less than 30 completed weeks the TEWL values were considerably higher than in infants near or at term. Thus during the first day of life TEWL was 15 times higher in the infants born after 25 weeks of gestation than in the full term infants.

DISCUSSION

It is difficult to define what is an acceptable loss of weight and water during the first days

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- 15 Sedin B Hammarlund K Nilsson G E &
Oberg P Å Evaporative water loss in full term
and premature newborn infants *Abstract no 47*
Sixth Nordic Congress of Perinatal Medicine Oulu
15-17/9 1977
- 16 Sedin B Hammarlund K Nilsson G E &
Oberg P Å The influence of environment activity
and gestational age on evaporative water loss *Proc*
Annual Meeting of European Society for Pediatric
Research no 51 Turku 25-29/6 1978
- 17 Stevenson J G Fluid administration in the asso-

ciation of patent ductus complicating respiratory
distress syndrom *J Pediatr* 90(?) 757 1977

- 18 Thomas B H Hyperosmolality and intraventricu-
lar haemorrhage in premature babies *Acta Paediatr*
Scand 65 4 9 1976

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poration are very high especially at low ambient relative humidities (cf. Fig. 2). The fact that the water loss by evaporation from the skin was increased by 100% in infants born after a gestational period of 26 completed weeks when the ambient relative humidity was reduced from 60 to 20% emphasizes the enormous importance of keeping the air in the incubator well humidified.

The high transepidermal water loss in preterm infants may be at least partly explained by their thin epidermal layer and their numerous superficial skin vessels. Very little is known about other factors that may be involved in the passage of water through the skin of newborn preterm infants.

CONCLUSIONS

This study shows that during the first day of life

1. There is a linear relation between the ambient humidity and the evaporation rate from the skin surface in preterm and full term infants.

2. The evaporation rate from the skin is higher in preterm infants than in term infants.

3. Susceptibility to changes in ambient humidity increases with decreasing gestational age.

4. The water loss by evaporation from the skin is increased by 100% in infants born after very short gestational periods when the ambient relative humidity is changed from 60 to 20%.

5. There is an exponential relationship between transepidermal water loss and gestational age.

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REFERENCES

1. Dubowitz I. M. S., Dubowitz V. & Goldberg C. Clinical assessment of gestational age in the newborn infant. *J. Pediatr.* 77: 1, 1970.
2. Fanaroff A. A., Wald M., Gruber H. S. & Klaus M. H. Insensible water loss in low birth weight infants. *Pediatrics* 50: 236, 1972.
3. Finnstrom O. Studies on maturity in newborn infants. IX. Further observations on the use of external characteristics in estimating gestational age. *Acta Paediatr. Scand.* 66: 601, 1977.
4. Friis Hansen H. Changes in body water compartments during growth. *Acta Paediatr. Scand. Suppl.* 110, 1956.
5. Hammarlund K., Nilsson G. E., Öberg P. Å. & Sedin G. Transepidermal water loss in newborn infants. I. Relation to ambient humidity and site of measurement and estimation of total transepidermal water loss. *Acta Paediatr. Scand.* 66: 551, 1977.
6. Hammarlund K., Nilsson G. E., Öberg P. Å. & Sedin G. Transepidermal water loss in newborn infants. II. Relation to activity and body temperature. *Acta Paediatr. Scand.* 68: 371, 1979.
7. Hammarlund K. & Sedin G. Evaporative water loss in preterm newborn infants. Abstract no. 197. *Sixth European Congress of Perinatal Medicine*, Vienna 29/8-1/9, 1978.
8. Heud W. C., Grebin B. & Winters R. W. The stabilization of disorders of water, electrolyte and acid base metabolism in newborn infants under intensive care. In H. Abramson (ed.) *Resuscitation of the newborn infant*. The Mosby Company, St. Louis, 1973, p. 240.
9. Hey E. N. & Katz G. Evaporative water loss in the newborn baby. *J. Physiol.* 200: 605, 1969.
10. Karlberg P. & Proliss A. Clinical evaluation of similarities and dissimilarities between the two city surveys. In F. Falkner (ed.) *Fundamentals of mortality risks during the perinatal period and in infancy*. Monogr. Paediatr. 9: 165, Karger, Basel, 1977.
11. MacLaurin J. C. Changes in body water distribution during the first two weeks of life. *Arch. Dis. Child.* 41: 286, 1966.
12. Nilsson G. E. Measurement of water exchange through skin. *Med. Biol. Eng. Comput.* 15: 209, 1977.
13. Nilsson G. E. On the measurement of evaporative water loss. Methods and clinical applications. *Länköping Studies in Science and Technology. Dissertations no. 11 and Linköping University Medical Dissertations no. 48*. Linköping, 1977.
14. Nilsson G., Sedin G. & Öberg A. A transducer for measurement of evaporation from the skin.

ARTERIAL BLOOD PRESSURE ELEVATIONS DURING MOTOR ACTIVITY AND EPILEPTIC SEIZURES IN THE NEWBORN

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ABSTRACT Lou H C and Friis-Hansen B (Department of Neurology Roskilde Hospital and the Department of Neonatology Rigshospitalet Copenhagen Denmark) Arterial blood pressure elevations during motor activity and epileptic seizures in the newborn *Acta Paediatr Scand* 68 803 1979.—In nine distressed newborn infants mean aortic blood pressure and motor activity were recorded continuously during the first two or three days of life. Six of the infants had been asphyxiated at birth the remainder having idiopathic respiratory distress only. The results showed that mean arterial blood pressure varies synchronously with motor activity reaching maximum values much higher than previously suspected about 90 or 100 mmHg were recorded in several infants. In three cases focal and/or generalized epileptic seizures occurred during the recording. It was found that in these circumstances too blood pressure increases dramatically even if the motor component of the seizure is insignificant.

KEY WORDS Hypertension motor activity epileptic seizures neonates intracranial bleedings

Recent evidence suggests that the arterial blood pressure may play a decisive role in the development of intraventricular bleeding in the newborn (1, 2). Most reports on the arterial blood pressure in newborn have focused on the resting blood pressure. However Moss et al (3) and Gupta et al (4) have found that feeding and crying is associated with blood pressure increases of up to 25 mmHg. The effects of general motor activity as well as

handling and caring procedures are not well documented and blood pressure changes during epileptic seizures have not been studied at all in the newborn. The present study was designed to obtain such information.

PATIENT GROUP

The patient group consisted of 9 consecutive infants with varying degrees of distress requiring monitoring with an intra arterial catheter. 6 had been asphyxiated at birth.

Table 1 Patient group

Patient no	Sex	Birth weight (g)	Gest age (weeks)	Apgar score		IRDS	MABP at rest (awake) mmHg			Maximum MABP mmHg		
				1 min	5 min		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
1/1	M	880	8	5	6	-	45	50	60	75	90	95
11/1	M	1700	39	9	10	+	35	35		55	55	
11	F	070	33	1		-	70	40		60	80	
12	M	040	3		9	+	45	55		55	85	
14	M	150	35	1	8	-	40	45	45	60	90	90
14/1	F	400	38	7	10	-	40	40		70	80	
20	F	900	36	10	10	+	60	65	65	100	100	110
21	M	900	40	3	4	-	40	40		70	80	
23	F	3000	39	4	6	-	40	40	40	100	75	80

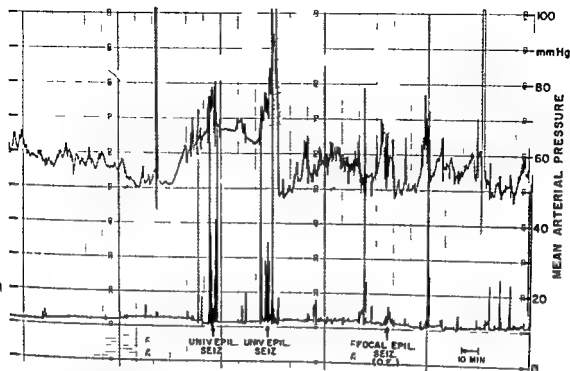


Fig 3 MABP increases from 50 to 95 mmHg are seen during generalized epileptic seizures (Patient 253)

were considerably higher than previously realized 55–110 mmHg (mean 86). All patients had marked increases synchronously with increases in motor activity (Fig 1) whether spontaneous (spont MA) or induced by the nursing procedure (suction (suct)) feeding handling observation procedure (obs proc) physiotherapy (phys) etc.

In 3 of the patients (253, 270 and 350) seizure activity was present during the recording. Patient 350 had brief generalized tonic-clonic convulsions as well as focal myoclonic seizures in the right leg. The EEG was characterized by sharp waves and spikes bitemporally and a background activity of 5–6 Hz with admixture of 9–10 Hz as well as 1½–3 Hz activity. The seizures were associated with an increase in MABP from about 40 to 80 mmHg. Patient 270 had periodic proxioms of up to 6 sec duration with 4–5 Hz activity and spike potentials in the EEG. These episodes were associated with apnoea and in some instances

slight tonic convulsions of upper extremities as well as sharp increases in MABP (Fig 2).

Patient 253 had focal myoclonic jerks of the upper extremities (focal epil seiz) as well as a few generalized tonic-clonic convulsions (univ epil seiz) of short duration during the first day of life. The convulsions ceased with phenobarbitone treatment. Repeated EEGs were normal. Fig 3 shows the very dramatic increase in MABP during seizures, especially during one of the generalized seizures with MABP reaching values of about 95 mmHg.

DISCUSSION

The principal finding of the present study has been the demonstration of the instability of MABP. High values are reached during spontaneous motor activity, manipulation of the infant and during seizures, whether or not associated with motor phenomena. The maximum MABP was in most cases about 100 mmHg.

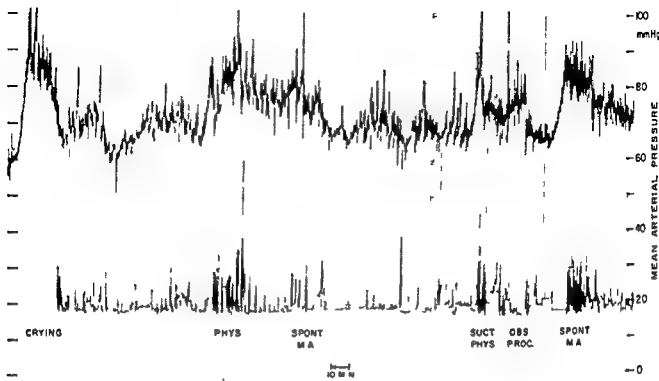


Fig. 1 Increases of MABP from 60 or 65 mmHg to 100 mmHg are seen to occur synchronously (upper curve) with registered motor activity (lower curve) (During the vigorous movements of crying the strain gauge is over stretched) (Patient 222)

(Apgar score below 7/1 min) and 3 had idiopathic respiratory distress syndrome. 6 had a birth weight below 2500 g (Table 1).

METHODS

Intra aortic mean pressure was recorded continuously by means of an end hole umbilical artery catheter with the tip in the thoracic aorta. The catheter was connected through a rigid polyethylene tube and a flushing valve to a transducer (Statham®) placed on the same level. The arterial blood pressure was electronically integrated with a time constant of 10 sec. and the mean arterial blood pressure (MABP) continuously recorded with a paper speed of 1 mm/min. In this way artifacts were minimized. The recording started 2–3 hours after birth and was continued throughout the first 2 or 3 days of life. The motor activity of the neonate was recorded synchronously by means of a strain gauge tied around the left leg (Medimatic®).

RESULTS

The principal findings are shown in the table. The MABP at rest was in accordance with other studies found to be in the range of 30–60 mmHg (mean 45) during the first days of life. However, the maximum MABPs recorded

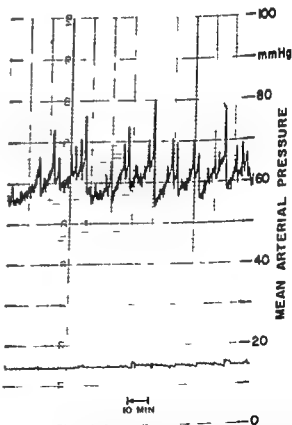


Fig. 2 Sharp increases of MABP from 55 to 80 mmHg are observed during subtle seizures consisting of apnoea with or without accompanying myoclonic jerks of the upper extremities. No motor activity is registered in the lower extremities (Patient 270)

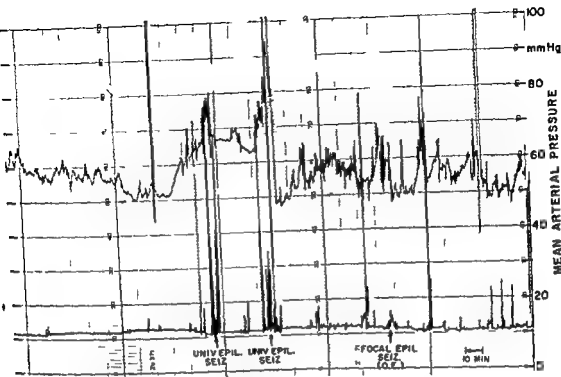


Fig 3 MABP increases from 50 to 95 mmHg are seen during generalized epileptic seizures (Patient 253)

are considerably higher than previously alized 55-110 mmHg (mean 86). All patients had marked increases synchronously with increases in motor activity (Fig 1) whether spontaneous (spont MA) or induced by the nursing procedure (suction), feeding, handling, observation procedure (obs proc), physiotherapy (phys), etc.

In 3 of the patients (253, 270 and 350) seizure activity was present during the recording. Patient 350 had brief generalized tonic-clonic convulsions as well as focal myoclonic seizures in the right leg. The EEG was characterized by sharp waves and spikes bilaterally and a background activity of 5-6 Hz with admixture of 9-10 Hz as well as 14-15 Hz activity. The seizures were associated with an increase in MABP from about 40 to 80 mmHg. Patient 270 had periodic paroxysms of up to 6 sec duration with 4-5 Hz activity and spike potentials in the EEG. These episodes were associated with apnoea and in some instances

slight tonic convulsions of upper extremities as well as sharp increases in MABP (Fig 2).

Patient 253 had focal myoclonic jerks of the upper extremities (focal epileptic seizure) as well as a few generalized tonic-clonic convulsions (univ. epileptic seizure) of short duration during the first day of life. The convulsions ceased with phenobarbitone treatment. Repeated EEGs were normal. Fig 3 shows the very dramatic increase in MABP during seizures, especially during one of the generalized seizures with MABP reaching values of about 95 mmHg.

DISCUSSION

The principal finding of the present study has been the demonstration of the instability of MABP. High values are reached during spontaneous motor activity, manipulation of the infant, and during seizures, whether or not associated with motor phenomena. The maximum MABP was in most cases about 100 mmHg.

higher than MABP at rest and increases of 50% or more were recorded many times during the day in all patients. This effect is probably due to increased sympathetic tone.

The variability of MABP in the newborn is greater than previously suspected. The dramatic increases even in small prematures may be responsible for the often fatal intracranial bleeding.

It has been shown recently that such bleeding originates in the capillaries of the germinal matrix (1) and that the capillaries are unprotected by normal autoregulation in the distressed newborn (2). In addition the capillaries are not supported by the firm glial membranes of the more mature brain (5). Thus the delicate capillary wall of the distressed premature infants is completely exposed to the large increases in MABP seen even in the first days of life.

REFERENCES

1. Hambleton G & Wigglesworth J S. Origin of intraventricular haemorrhage in the preterm infant. *Arch Dis Child* 51: 651, 1976.
2. Lou H C, Lassen N A & Friis Hansen B. Impaired autoregulation of cerebral blood flow in the distressed newborn. *J Pediatr* 94: 118, 1979.
3. Moss A J, Duffie E R & Emmanouilides G. Blood pressure and vasomotor reflexes in the newborn infant. *Pediatrics* 32: 175, 1963.
4. Gupta J M & Scopes J W. Observations on blood pressure in newborn infants. *Arch Dis Child* 40: 63, 1965.
5. Prestige M C. Axon and cell numbers in the developing nervous system. *Br Med Bull* 30: 107, 1974.

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NEONATAL CONVULSIONS

Incidence and Causes in the Stockholm Area

MARGARETA ERIKSSON and ROLF ZETTERSTRÖM

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ABSTRACT Eriksson M and Zetterstrom R (Department of Paediatrics, Karolinska Institute, St. Goran's Children's Hospital, Stockholm, Sweden). Neonatal Convulsions: Incidence and Causes in the Stockholm Area. *Acta Paediatr Scand* 68: 807, 1979.—The causes and short term prognoses of neonatal convulsions in infants less than four weeks of age were studied in 77 full term infants born in Stockholm in 1970-1976. In half of the infants (48%) hypoxia was considered to be the probable main etiology, while infection and metabolic disease including hypoglycemia and hypocalcemia were the next commonest cause (12% for each condition). The etiology was unknown in 29% of the infants although 15 of those included in this group had other additional diagnoses. The total mortality was 13%. At one year of age 19 of the surviving 64 infants (30%) had severe psychomotor retardation. Of 11 infants with normal mental development at 12 months of age 6 had cerebral palsy and 5 epileptic seizures. Thirty-four (53%) of the infants still had no signs of sequelae. The poorest prognosis was found in the group with hypoxia as the main probable etiology. The incidence of neonatal convulsions was 1.5 per 1000 full term deliveries. In a similar study from Gothenburg which was performed 10 years earlier the incidence was 3.7 per 1000. Corresponding figures for perinatal mortality rate were 13.5 and 23.8.

KEY WORDS Newborn convulsions, etiology, prognosis, incidence.

There is evidence that an increased concern for maternity and infant care has led to a reduced neonatal mortality and morbidity (8, 21). In Sweden, this has resulted not only in a decline in perinatal mortality, but it has also been accompanied by a significant decrease in the incidence of cerebral palsy during the last decades (12). The decrease in cerebral palsy mainly relates to perinatal and neonatal as well as to acquired forms and has been attributed to better obstetric and neonatal care. Against this background, we decided to study another symptom which is frequently related to the quality of prenatal and neonatal and obstetric care—i.e. neonatal convulsions (7).

The reported incidence of neonatal convulsions varies considerably in different parts of the world. Several prospective studies and review articles give figures ranging from 3.8 to 14.0 per 1000 deliveries (4, 7, 14, 19, 20). In Sweden, an incidence of 3.7/1000 term infants was reported from Gothenburg for 1960-1962

(23). The possible etiologies for convulsions and their relative frequencies also vary.

In several recent studies the mortality rate and the incidence of sequelae in newborns with convulsions appear to have changed (15, 18, 20). Until 1970 the reported mortality was about 40% and one third of the surviving infants showed sequelae. A lower mortality rate has been reported in later reviews (15-20%) on the other hand, a large number of the surviving infants are handicapped (40%). The presence of sequelae correlates with the possible etiology of the seizures as well as with its severity and the age of the infant when convulsions develop. The poorest prognosis has been found in infants with perinatal asphyxia and early symptoms (13, 21).

MATERIAL AND METHODS

Information has been collected concerning 77 consecutive infants with neonatal convulsions who were seen at St. Goran's Children's Hospital during a seven year period

higher than MABP at rest and increases of 50% or more were recorded many times during the day in all patients. This effect is probably due to increased sympathetic tone.

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MATERIAL AND METHODS

Information has been collected concerning 77 consecutive infants with neonatal convulsions who were seen at St. Goran's Children's Hospital during a seven year period.

Table 1 Probable etiology of neonatal convulsions in 77 infants

Probable main etiology	Infants	
	No	%
Hypoxia	37	48
Infection	9	12
Metabolic hypoglycemia	5	6
Metabolic hypocalcemia	2	3
Other defined metabolic causes	2	3
Unknown	22	29
No additional diagnosis	7	
Unknown other disease	15	

(1970-1976). The infants came both from the referral area of its three maternity hospitals (33/976 deliveries) and from other pediatric hospitals for intensive care. Full term infants (≥ 37 gestational weeks) with clonic or tonic seizures less than four weeks of age were included in the study. Ten pre-term infants presenting during the same period were not included because in some cases it was difficult to diagnose seizures in these infants. Gestational age was calculated on the basis of external and neurological maturity characteristics.

The following data were obtained from case records: information on pregnancy, delivery (including signs of fetal distress), Apgar score at 1 and 5 min, blood glucose, serum calcium and magnesium as well as other diagnoses and investigations relevant to the seizure disorder. The infants were classified into different groups with regard to the most probable cause of the seizure. The diagnosis of hypoglycemic and hypocalcemic convulsions was made when the criteria of a low blood glucose (<1.7 mmol/l), serum calcium (<1.9 mmol/l) and cessation of seizures upon administration of glucose or calcium were fulfilled (2, 6).

Most infants were followed in a special high risk clinic up to the age of one year. In a few cases information was obtained by interview and examination of records at the child health centre.

RESULTS

The incidence of neonatal convulsions in our referral area (i.e. 49 of the 77 infants) was

estimated as 1.5/1000 full term deliveries. Of the 77 full term infants 17 (22%) had a birth weight two standard deviations below the mean. The corresponding figure for Sweden in 1974 was 2.2% (according to the birth register at the National Board of Health and Welfare).

Table 1 shows the distribution of the infants according to the most probable cause. The 15 infants with an additional diagnosis included 4 with congenital malformations, 3 with hyperbilirubinemia (bilirubin 270-500 mmol/l), 3 small for gestational age babies, one neonatal polycythemia (packed red cell volume 76%), one maternal diabetes, one hemolytic disease from Rh immunization and one neonatal hepatitis. The total mortality was 10/77 (13%).

The majority of infants had their first seizure before three days of age. An early onset was common in asphyxia and a late onset was common in septicemia. One third of the infants had mild convulsions (i.e. less than 10 min duration). No difference in severity between the groups was found except in otherwise normal infants with convulsions of unknown etiology where multiple prolonged seizures were always noted.

Table 2 shows the biochemical changes in all infants grouped according to the most probable etiology. As can be seen, low blood glucose and serum calcium concentrations were found in several infants with asphyxia. Asphyxia was also occasionally present in groups with other etiologies.

The prognosis at one year of age which is shown in Table 3 was found to be related to

Table 2 Hypoglycemia and/or hypocalcemia in 77 infants with neonatal convulsions

Probable main etiology	No	Blood glucose <1.7 mmol/l	Serum calcium <1.9 mmol/l
Hypoxia	37	12	3
Infection	9	1	1
Metabolic hypoglycemia	5	5	
Metabolic hypocalcemia	2		2
Other defined metabolic causes	2		1
Unknown no additional diagnosis	7	0	0
Unknown other disease	15	4	2

Table 3 Follow up at one year of age of infants with neonatal convulsions

Probable main etiology	No of infants	Mentally normal		No apparent sequelae	Obvious psychomotor retardation	Died
		CP	Seizures			
Hypoxia	37	6	3*	16	6	6
Infection	9			3	4	2
Metabolic						
Hypoglycemia	5			2	3	
Hypocalcemia	2			2		
Other	2					2
Unknown						
No additional diagnosis	7		2	2	3	
Other disease	15			9	3	3

One infant in each group with severe retardation survived the neonatal period but died before one year of age
Only with birth temperature

the seventy of the convulsions. In 11 children with a few short seizures presumably due to hypoxia no sequelae occurred. Three however had febrile seizures starting at 6-9 months of age. In other groups with mild convulsions severe retardation was found in one child with meningitis and in one with symptomatic hypoglycemia. Six children with cerebral palsy (varying severity of spastic diplegia) were considered to be mentally normal at one year of age.

DISCUSSION

The incidence of neonatal convulsions was 1.5/1000 full term deliveries in our referral area for the years 1970-1976. This figure is considerably lower than the rates reported from other countries, i.e. 3.7-14/1000 deliveries. The incidence was found to be 3.7/1000 in Gothenburg which is Sweden's second largest city during the years 1960-1962 (23). One explanation for the difference between various studies may be related to the selection of patients, especially with respect to the inclusion of pre-term infants. For example Hopins (incidence 3.8/1000) included all infants with a birth weight above 1500 g while Rose & Lombroso studied those with a weight of 2500 g and more (14, 19).

The reason for the low incidence of neonatal convulsions in our study and the declining in

cidence in Sweden is probably multifactorial. As mentioned in the introduction the low incidence in the Oxford area compared to Edinburgh and Manchester was tentatively attributed to better prenatal and obstetric care (7). In Sweden during the same period the decreases in perinatal mortality and in cerebral palsy, a sequelae of perinatal complications have also been attributed to better perinatal care (9, 12).

In this review the same grouping as in most other studies (i.e. according to the most probable etiology) has been used. There is a demonstrable difference between these studies even if the grouping sometimes is difficult and may be open to discussion. In the majority of the studies however perinatal hypoxia and birth trauma are the most common causes. In our study we found that approximately 50% of the infants were subjected to perinatal hypoxia. Lower relative incidences have been reported from England and the USA. If however a comparison is made with respect to the total number of affected infants the rate of 13% due to asphyxia reported by Keen & Lee (15) from Manchester corresponds to 1.5/1000 deliveries which is still higher than the figure of 0.7/1000 in this Swedish review. Differences in the percentage due to metabolic disease have also been reported. Symptomatic hypoglycemia however has been found in the same relative incidence of 5-10% in most

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Hypocalcemia	~					7
Other	~					
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No additional diagnosis	7		2	2	3	
Other disease	13			9	3	1

One infant in each group with severe retardation survived the neonatal period but died before one year of age

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studies except in that of Hopkins (14) from Australia where it was demonstrated in one third of the cases. However it remains unclear whether the criteria of symptomatic hypoglycaemia which were used by Hopkins were the same as those used by other workers (2). Another explanation of the discrepancy might be a difference in the number of preterm infants and variations in feeding habits during the years concerned. We found symptomatic hypoglycaemia in association with both asphyxia and infection but in these instances the seizures did not respond to glucose administration. If these infants are included hypoglycaemia was found in almost one third of the cases and in the same percentage is found by Hopkins (14). In studies from Scandinavia it has been found that asymptomatic hypoglycaemia very often is associated with a number of pathological conditions in newborn infants and that only a minority of the infants with hypoglycaemia develop convulsions (10, 11, 16).

A high incidence of hypocalcaemia is a possible cause of neonatal convulsions has been reported from England and the USA (6, 18, 19). It is possible however that the requirement of cessation of convulsions upon administration of calcium was not mandatory in all cases. In our study we did not find a low serum magnesium in a single case as a possible explanation of failure to respond to calcium administration. However a study by Cockburn *et al.* (6) showed that at the time of convulsions low calcium values could be found in the CSF; moreover the establishment of equilibrium between the serum and the CSF could take some time after the administration of calcium consequently a prompt cessation of convulsions could not be expected. Even if the few cases of a low serum calcium in the other groups are taken into consideration the absolute as well as the relative frequencies are low. In the Edinburgh study (4) with an incidence of 14.0/1000 live births disturbances in calcium or magnesium metabolism were thought to be the causative factor in 55% of

the cases. This in turn was suggested to be due to a higher phosphate content in cow's milk in comparison with breast milk since convulsions were less often seen in infants fed on a modified cow's milk formula with a low phosphate content (6).

Infections have been found to occur in about 10% of all studies of convulsions in which cases meningitis has usually been present. The group with unknown etiology will naturally vary in different studies. Some infants having a familial type of convulsion with a favourable prognosis have been included in this group (3, 5).

The prognosis of neonatal convulsions has been investigated in a limited number of follow up studies (7, 15, 18, 20). It will of course vary with the etiology. Before 1970 a total early mortality of 40% sequelae in 19% and normal development in 41% of the infants were reported. More recent studies have shown a lower mortality but also a higher percentage of survivors with sequelae while the percentage of infants with normal development has remained fairly constant (53%). We found an overall mortality of 13% and handicaps at the age of one year to be 53%. Minor abnormalities may however be diagnosed later and long term follow up is important. It is also important as mentioned earlier to analyse the figures in individual groups.

A high mortality rate (43%) and sequelae in half of the surviving children has been reported in convulsions associated with asphyxia (15, 21). We found a lower mortality rate (14%) but on the other hand more children with handicaps (41%) thus making the total morbidity about the same. The unfavourable prognosis of convulsions in infants with asphyxia has been observed earlier in a Swedish study of children born 1943–1949 in which eight of nine children with convulsions had sequelae (13). The poorest prognosis is probably seen in this group with asphyxia and seizures (21).

In the two cases we found with hypocalcaemia the prognosis was good as others have

also noted. In hypoglycemia on the other hand the prognosis varies. In a follow up of children with hypoglycemia the poorest prognosis was found in children with convulsions (10, 11, 16). Three of five children in our follow up were severely retarded. We also found hypoglycemia as an additional symptom to be a bad prognostic sign in the other groups.

One important finding is that convulsions per se in association with infections do not however lead to more sequelae than was reported in a follow up of children with neonatal septicemia (1). As others have also demonstrated the short term prognosis is favourable in children with convulsions of unknown etiology except for a tendency to seizures later in life (3, 5).

The incidence of neonatal convulsions in full term live births was much lower in this study of infants born 1970-1976 (1.5/1000) than in a similar study from Gothenburg from 1960-1962 with an incidence of 3.7/1000 (23). The decrease may be attributed to improved obstetric and neonatal care. The difference between the incidence of neonatal convulsions in the Gothenburg and Stockholm series is rather similar to corresponding figures for perinatal mortality rate which was 23.8 in Gothenburg 1960-1962 and 13.5 in Stockholm 1970-1976.

Even if neonatal convulsions are still a sign which may indicate a serious condition with a high morbidity and risk for later sequelae the mortality rate is lower than in previous studies. The children in this study however are still very young and it is important to have a long term follow up with respect to later and less serious sequelae.

REFERENCES

1. Alfén G, Bergqvist M, Bolme P & Eriksson M. Longterm follow up of neonatal septicemia. *Acta Paediatr Scand* 67: 769 1978.
2. Beard A, Cornblath M, Gentz J, Kellum M, Persson B, Zetterstrom R & Haworth J C. Neonatal hypoglycemia. A discussion. *J Pediatr* 79: 314 1971.
3. Bjerre I & Corelius E. Benign familial neonatal convulsions. *Acta Paediatr Scand* 57: 557 1968.
4. Brown J K. Convulsions in the newborn period. *Dev Med Child Neurol* 15: 873 1973.
5. Carton D. Benign familial neonatal convulsions. *Neuropediatrics* 11: 167 1978.
6. Cockburn F, Brown J K, Belton N R & Forfar J O. Neonatal convulsions associated with primary disturbance of calcium, phosphorus and magnesium metabolism. *Arch Dis Child* 48: 99 1973.
7. Dennis J. Neonatal convulsions: aetiology, late neonatal status and long term outcome. *Dev Med Child Neurol* 20: 143 1978.
8. Elliot K & O'Connor M. *Major Mental Handicap and Costs of Prevention*. Ciba Foundation Symposium 59 (New Series). Elsevier Excerpta Medica, Amsterdam 1978.
9. Ericson A, Eriksson M & Zetterstrom R. Analysis of perinatal mortality rate in the Stockholm area. *Acta Paediatr Scand*. In press.
10. Fluge G. Neurological findings at follow up in neonatal hypoglycaemia. *Acta Paediatr Scand* 64: 69 1975.
11. Gentz J, Persson M & Zetterstrom R. On the diagnosis of symptomatic neonatal hypoglycemia. *Acta Paediatr Scand* 58: 449 1969.
12. Hagberg H, Hagberg G & Olow I. The changing panorama of cerebral palsy in Sweden 1954-1970. *Acta Paediatr Scand* 64: 187 1975.
13. Hellstrom M & Jonsson B. Late prognosis in asphyxia neonatorum. *Acta Paediatr Scand* 47: 398 1953.
14. Hopkins I. Seizures in the first week of life. A study of aetiological factors. *Med J Aust* 2: 647 1977.
15. Keen J H & Lee D. Sequelae of neonatal convulsions. *Arch Dis Child* 48: 54 1973.
16. Korvisto M, Blanco-Sequeiros M & Krause U. Neonatal symptomatic and asymptomatic hypoglycaemia. A follow up study of 151 children. *Dev Med Child Neurol* 14: 603 1972.
17. McDonald R. Purulent meningitis in newborn babies. *Clin Pediatr (Phila)* 11: 450 1972.
18. McNerny T & Schubert W. Prognosis of neonatal seizures. *Am J Dis Child* 117: 761 1969.
19. Rose A & Lombroso C. Neonatal seizure states. A study of clinical, pathological and electroencephalographic features in 137 full term babies with a long term follow up. *Pediatrics* 45: 404 1970.
20. Volpe J. Neonatal seizures. *N Engl J Med* 289: 413 1973.
21. Volpe J. Perinatal hypoxic ischemic brain injury. *Pediatr Clin North Am* 23: 383 1976.
22. Wallace H. Status of infant and perinatal morbidity and mortality. *Public Health Rep* 93: 386 1978.
23. Zetterstrom R. Neonatal chemistry. In H. E. Whipple & J. A. Sterling (eds). *Fetal and Infant Liver Function and Structure*. Ann NY Acad Sci 111. New York 1963. p. 537.

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SODIUM EXCRETION IN RELATION TO SODIUM INTAKE AND ALDOSTERONE EXCRETION IN NEWBORN PRE TERM AND FULL TERM INFANTS

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ABSTRACT Aperia A Broberger O Herin P and Zetterstrom R (Department of Paediatrics Karolinska Institutet St Goran's Children's Hospital and Huddinge Hospital Stockholm Sweden) Sodium excretion in relation to sodium intake and aldosterone excretion in newborn pre term and full term infants *Acta Paediatr Scand* 68 813 1979 —The importance of aldosterone for the control of salt balance has been examined in pre term infants (gestational age 28-34 weeks) and in full term infants The post natal age has varied from 2-21 days Eight hour urinary specimens have been analysed with regard to sodium potassium and aldosterone The daily sodium intake has been recorded following determination of milk intake and analyses of sodium in breast milk Due to variations of sodium content of breast milk the daily sodium intake in pre term infants was lower than in full term infants during the first 10 days of life The sodium excretion was significantly higher in pre-term infants than in full term infants during the first six days of life During the first week of life the sodium balance is negative in pre-term infants and positive in full term infants Aldosterone excretion is high during the first week of life and increases further from the 2nd to the 3rd week of life in both pre-term and full term infants The correlation between aldosterone excretion and urinary potassium/sodium quotient is 0.87 in full term infants 0.57 in pre-term infants aged 13-20 days and does not exist in pre term infants aged 2-10 days It is suggested that the high sodium excretion in newborn pre term infants can in part be explained by an unresponsiveness to aldosterone at this developmental stage

KEY WORDS Neonatal Na balance neonatal aldosterone excretion renal maturation aldosterone responsiveness renal function in pre term infants renal function in full term infants

Newborn infants have a blunted natriuretic response to salt loading (3) which suggests a reduced tolerance to salt in the neonatal period It has therefore been recommended that the daily sodium intake administered orally or parenterally to newborn infants should be kept lower than the daily sodium intake to older children This recommendation has been supported by the assumption that breast milk during the entire period of lactation contains about 5 mmol/l of Na (18) There are however several observations mainly in pre term infants that are at variance with the recommendation that the daily sodium intake should not exceed the expected sodium intake when infants are fed breast milk containing 5 mmol/l of Na Newborn pre term infants receiving parenteral fluid with a

sodium concentration of 10 and 20 mmol/l consistently develop a negative sodium balance (4) Newborn full term infants receiving parenteral fluids develop a negative sodium balance when the sodium concentration of the fluid is 10 mmol/l but generally not when it is 20 mmol/l Newborn pre term infants are reported to have a higher basal sodium excretion than newborn full term infants (5 6 13) and they frequently develop hyponatremia (7)

This study has been designed to describe the postnatal changes in sodium balance in pre term and full term infants with an uncomplicated postnatal course A record has been kept of the sodium intake and output In order

Dedicated to Andrea Prader a great scientist in the field of paediatrics on the occasion of his 60th anniversary

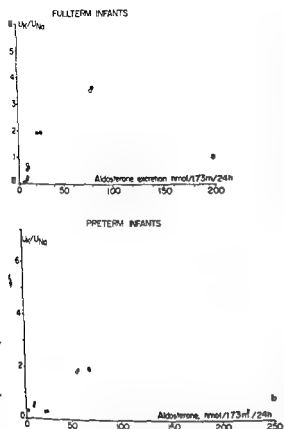


Fig 3a and b Relationship between aldosterone excretion and urinary K/Na ratio in full term infants (a) and pre term infants (b). \bullet infants with gestational age 78–34 weeks and aged 1–16 days that are not reported in previous figures and tables are included in b. Filled circles represent infants aged 1–10 days and unfilled circles infants aged 1–1 days.

In newborn full term infants (Fig 1a) the urinary sodium excretion was relatively low during the second to fourth day of life increased slightly but insignificantly towards the end of the first week of life and then fell within the same range at three weeks of life as at two to four days of life. The sodium concentration of breast milk was four times higher during the first 10 days after delivery than later during lactation (2). This resulted in a relatively high sodium intake during the first 10 days of life. During the same period the intake of sodium in daytime well exceeded the excretion in the full term infants. In pre term infants urinary sodium excretion was relatively high during the first days of life (Fig 1b). It gradu-

ally fell during the first week of life and reached the range observed in full term infants at the age of nine or ten days. The differences between pre term and full term infants were significant during day 3–4 ($p < 0.001$) and day 5–6 ($p < 0.01$). All pre term infants included in this study received pooled breast milk during the first 10 days of life. The sodium content of this milk averaged 5 mmol/l and the sodium intake was therefore considerably lower in pre term than full term infants. In pre term infants the sodium excretion in daytime exceeded the sodium intake in daytime during the first five days of life. At three weeks of age the sodium intake in daytime exceeded the sodium excretion in daytime to about the same extent as in full term infants.

The postnatal changes in aldosterone excretion in pre term and full term infants are illustrated in Fig 2. During the first three weeks of age the average aldosterone excretion in pre term and full term infants ranged from 18 to 105 $\mu\text{mol}/173\text{ m}^2\text{ S A/day}$ which is higher than in adults where the normal range in this laboratory is 5.5 to 33 $\mu\text{mol}/173\text{ m}^2\text{ B S A/day}$. The postnatal pattern for aldosterone excretion was similar in pre term and full term infants. The excretion of aldosterone was generally higher in pre term than in full term infants but the difference was never of more than borderline significance ($p > 0.8$) during day 3–4 ($0.1 < p > 0.05$) during day 5–12. Aldosterone excretion was highest at 3 weeks of age.

It is well established that aldosterone affects tubular sodium and potassium transport in opposite directions. An increase in the urinary potassium/sodium quotient has therefore generally been interpreted as secondary to an effect of aldosterone on the tubular transport mechanism (10). In newborn full term infants there is a good correlation ($r = 0.84$) between aldosterone excretion and urinary potassium/sodium quotient (Fig 3a). The relationship is the same in full term infants aged 0–10 days as in full term infants aged 11–20 days. In pre term infants aged 0–10 days there is no rela-

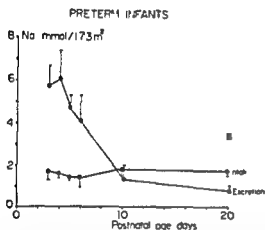
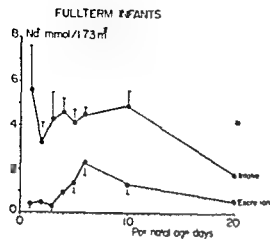


Fig. 1 *a* and *b* Average hourly Na^+ excretion and Na^+ intake at the time of the study in full term infants aged 1–21 days (*a*) and in pre term infants aged 3–21 days (*b*). Each filled circle represents the average of 4–9 observations. The bars represent S.E.M.

to elucidate some possible mechanisms responsible for the control of sodium balance in the neonatal period, the aldosterone excretion was also determined in most of the infants studied.

MATERIAL AND METHODS

35 pre term and 45 full term infants were studied on the Neonatal Ward of St. Goran's Children's Hospital and in the Obstetric Ward of St. Erik's Hospital.

In the pre term infants the gestational age ranged from 28 to 34 weeks. The postnatal course in these infants was regarded as uncomplicated since there was no need of assisted respiration, antibiotic treatment for infection or exchange transfusion. All pre term infants were kept in their incubators during the study. They were fed via a gastric tube.

In the full term infants the gestational age ranged from 39 to 41 weeks and the postnatal course was uncomplicated.

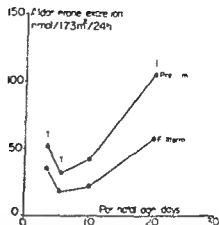


Fig. 2 Postnatal changes in aldosterone excretion in pre term and full term infants. Each filled circle represents the average of 4–10 observations. The bars represent S.E.M.

phicated. The studies were carried out in the Obstetric Ward and the infants were nursed by their mothers.

No mother was treated with diuretics during pregnancy. The gestational age of all infants was determined using the method of Dubowitz et al. (9).

Urine was collected for eight to nine hours from each infant by spontaneous voiding into a plastic bag. A tube was tightly fitted into the bag which allowed emptying of the bag without removing it. Urine collection was started and completed immediately after a voiding. In each case the urine collection was started between 7.30 and 9.30 a.m. During the study the infants were fed as usual, either by a gastric tube (pre term infants) or by breast feeding (full term infants).

The infants were either fed breast milk from their mothers (full term infants) or pooled breast milk from mothers in a later stage of lactation (pre term infants). The milk intake from 8 a.m. to 4 p.m. (in general 3 meals) was recorded. The sodium content of the milk was analysed in each individual case since it has been found to be higher during the first 2 weeks after delivery than later during lactation. (2) The milk intake of the full term infants was assessed by weighing them immediately before and after each meal. The average hourly sodium intake at the time of the study was calculated.

Analyses of sodium and potassium in the urine were made with a flame photometer. Analysis of aldosterone in the urine was made with a radioimmuno assay method (21).

Statistical analysis has been carried out with Student's *t* test.

RESULTS

Fig. 1 *a* and *b* illustrate the average Na^+ intake and output during the time of the study in infants aged three, four, five, six, eight, to ten and 18–21 days.

natal period (20). Studies in fetal guinea pigs have suggested that aldosterone is one factor of importance for the induction of this enzyme (11). It is of interest in this connection that the significant increase in aldosterone excretion which occurs from the first to the third week of life is not associated with a further fall in urinary sodium excretion and coincides with a period of rapid increase in the glomerular filtration rate (12), i.e. an increase of the work load to the renal tubules.

REFERENCES

1. Aperia A & Broberger U β microglobulin an indicator of renal tubular maturation and dysfunction in the newborn *Acta Paediatr Scand* In press
2. Aperia A, Broberger O, Hern P & Zetterstrom M Salt content in human breast milk during the three first weeks after delivery *Acta Paediatr Scand* In press
3. Aperia A, Broberger O, Thodenius K & Zetterstrom R Renal response to an oral sodium load in newborn full term infants *Acta Paediatr Scand* 61: 670 1977
4. Aperia A, Broberger O, Thodenius K & Zetterstrom R Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy *Acta Paediatr Scand* 64: 775 1975
5. Arant B S Developmental patterns of renal functional maturation compared in the human neonate *J Pediatr* 97: 85 1978
6. Broberger U & Aperia A Renal function in idiopathic respiratory distress syndrome *Acta Paediatr Scand* 67: 313 1978
7. Chance G W, Ruddle I C, Wilks D M, Roy R N, Park M & Ackerman I Postnatal growth of infants of <1.3 kg birth weight. Effects of metabolic a. idosis, of caloric intake and of calcium, sodium and phosphate supplementation *J Pediatr* 91: 787 1977
8. Dillon M J, Collins M E A, Rjagess J M & de Swiet M Plasma renin activity and aldosterone concentration in the human newborn *Arch Dis Child* 51: 437 1976
9. Dubowitz L M S, Dubowitz V & Goldberg C Clinical assessment of gestational age in the newborn infant *J Pediatr* 77: 1 1970
10. Ganong W F & Mulrow P J Rate of change in sodium and potassium excretion after ingestion of aldosterone into the aorta and renal artery of the dog *Am J Physiol* 195: 337 1978
11. Geloso J P, Delaval E, Geloso-Meyer A, Lelievre M Enzyme development and functional differentiation of fetal rat kidney. In *Ontogeny of renal function* 7th International Congress of Physiology, California Science, Paris 1977
12. Guignard J P, Torrado A, Dacunha O & Gauthier E Glomerular filtration rate in the first three weeks of life *J Pediatr* 87: 268 1975
13. Honour J W, Valman H B & Shackleton C G L Aldosterone and sodium homeostasis in full term infants *Acta Paediatr Scand* 66: 103 1977
14. Jorgensen P L The role of aldosterone in the regulation of (Na⁺ + K⁺) ATPase in rat kidney *J Steroid Biochem* 3: 181 1972
15. Krans M, Krecsek J & Popp M The development of aldosterone production by the adrenal glands of normally and prematurely weaned rats *Bol Neonat* 11: 338 1967
16. Kowarski A, Katz H & Migeon C J Plasma aldosterone concentration in normal subjects from infancy to adulthood *J Clin Endocrinol Metab* 38: 489 1974
17. Merlet Benichou C & de Rouffignac D Renal clearance studies in fetal and young guinea pigs: effect of salt loading *Am J Physiol* 232: 178 1977
18. Renner F *Milch und Milchprodukte in der Ernährung des Menschen* Volkswirtschaftliches Verlag GmbH Kempfen-Verlag Th. Mann OHG Hildesheim 1974
19. Roy R N, Chance G W, Ruddle I C, Hill D E, Wilks D M & Sheepers J Late hyponatremia in very low birth weight infants (<1.3 kg) *Pediatr Res* 10: 56 1976
20. Schmidt V & Horster M Na/K activating ATPase activity maturation in rabbit nephron segments dissected in vitro *Am J Physiol* 233: F35 1977
21. Sealey J E, Buhler F R, Laragh J H, Manning E L & Brunner H R Aldosterone excretion *Circ Res* 31: 367 1972
22. Siegel S R, Fisher D A & Oh W Serum aldosterone concentrations related to sodium balance in the newborn infant *Pediatrics* 53: 410

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tionship between the aldosterone excretion and the urinary potassium sodium quotient. In pre term infants aged 10–20 days the urinary potassium sodium quotient is consistently higher than in younger pre term infants and there is a correlation between aldosterone excretion and the urinary potassium sodium quotient ($r=0.57$).

DISCUSSION

The findings of the present study explain the susceptibility of pre term infants to hyponatraemia (19). During the first week of life pre term infants have both larger urinary sodium losses and a lower sodium intake than full term infants. The relatively low salt intake of pre term infants during the first week of life has previously been overlooked when the salt balance of newborn infants has been discussed. Yet most pre term infants have been fed with pooled breast milk having a sodium concentration of 5 mmol/l or with a formula having a low sodium concentration. It has however been reported that sodium supplementation to pre term infants on this diet results in a significant increase of body length (7). This finding together with the above mentioned observations of the frequently occurring negative salt balance in pre term infants (4, 13, 22) strongly suggests that pre term infants who for some reason cannot be wholly fed with breast milk from their mothers should at least during the first two weeks of life receive sodium supplements so that the daily sodium intake/kg b.w. corresponds to the sodium intake of breast fed term infants of similar postnatal age.

High neonatal aldosterone production has previously been observed (8, 13, 16, 22) in man as well as in other species (15). The neonatal aldosterone production does not primarily appear to be monitored by changes in salt balance. During the first week of life there is a slight fall in aldosterone excretion which can be explained by a high sodium intake in the full term infants but this also occurred in

pre term infants who had an inadequate sodium intake. The aldosterone production appears to increase from the first to the third week of life not only in the newborn pre term infants who are in negative but also in the newborn full term infants who are in positive sodium balance. Since the postnatal pattern for aldosterone excretion is almost identical in newborn pre term and full term infants, birth appears to be an important regulator of aldosterone production in early postnatal life.

High sodium excretion in pre term infants has been reported in several previous studies (4, 5, 6, 13, 22). Since the glomerular filtration rate is lower in pre term than in full term infants (5), a high sodium excretion in newborn pre term infants must be attributed to relatively low tubular reabsorption of sodium. The 34th to the 36th gestational week appears to be a period of rapid development for renal tubular transport mechanism. The fractional reabsorption of β microglobulin (1) as well as of glucose and amino acids (5) increase adult levels during this period. Experimental studies in guinea pigs (17) have demonstrated that the fractional reabsorption of sodium is low during fetal life and increases rapidly at term. It has been suggested that the changes in sodium excretion in guinea pigs are at least partly monitored by aldosterone. In the present study it has been shown that aldosterone excretion is at least as high in newborn pre term infants with a high sodium excretion as in newborn full term infants with a low sodium excretion. The lack of relationship between aldosterone excretion and the urinary potassium sodium quotient in newborn pre term infants does however strongly suggest that infants born before the 34th gestational week have the renal tubular sensitivity for aldosterone low. This could be due either to a lack of receptors for aldosterone or to a deficiency of the sodium transporting enzymes sodium potassium activated ATPase which aldosterone will affect (14). Experimental studies in rabbits have shown that the ATPase activity is low in all tubular segments in the neo-

natal period (20). Studies in fetal guinea pigs have suggested that aldosterone is one factor of importance for the induction of this enzyme (11). It is of interest in this connection that the significant increase in aldosterone excretion which occurs from the first to the third week of life is not associated with a further fall in urinary sodium excretion and coincides with a period of rapid increase in the glomerular filtration rate (12) i.e. an increase of the work load to the renal tubules.

REFERENCES

1. Aperia A & Broberger U. β -microglobulin an indicator of renal tubular maturation and dysfunction in the newborn. *Acta Paediatr Scand*. In press.
2. Aperia A, Broberger O, Henn P & Zetterstrom R. Salt content in human breast milk during the three first weeks after delivery. *Acta Paediatr Scand*. In press.
3. Aperia A, Broberger O, Thodenius K & Zetterstrom R. Renal response to an oral sodium load in newborn full term infants. *Acta Paediatr Scand*. 61 (1972).
4. Aperia A, Broberger O, Thodenius K & Zetterstrom R. Renal control of sodium and fluid balance in newborn infants during intravenous maintenance therapy. *Acta Paediatr Scand*. 64 725 1975.
5. Arant B S. Developmental patterns of renal functional maturation compared in the human neonate. *J Pediatr*. 93 705 1978.
6. Broberger U & Aperia A. Renal function in idiopathic respiratory distress syndrome. *Acta Paediatr Scand*. 67 313 1978.
7. Chance G W, Ruddle J C, Willis D M, Roy R A, Park E & Ackerman J. Postnatal growth of infants of <1.3 kg birth weight. Effects of metabolic acidosis of caloric intake and of calcium, sodium and phosphate supplementation. *J Pediatr*. 91 787 1977.
8. Dillon M J, Gillin M E A, Rydness J M & de Swiet M. Plasma renin activity and aldosterone concentration in the human newborn. *Arch Dis Child*. 51 537 1976.
9. Dubowitz L M S, Dubowitz V & Goldberg C. Clinical assessment of gestational age in the newborn infant. *J Pediatr*. 77 1 1970.
10. Ganong W F & Mulrow P J. Rate of change in sodium and potassium excretion after injection of aldosterone into the aorta and renal artery of the dog. *Am J Physiol*. 195 337 1958.
11. Geloso J P, Delaval E, Geloso-Meyer A, Lehevre M. Enzyme development and functional differentiation of fetal rat kidney. In: *Ontogeny of renal function. 27th International Congress of Physiological Science*. Paris 1977.
12. Guignard J P, Torrado A, Dacunha O & Gauthier E. Glomerular filtration rate in the first three weeks of life. *J Pediatr*. 87 88 1975.
13. Honour J W, Valman H B & Shackleton C G L. Aldosterone and sodium homeostasis in full term infants. *Acta Paediatr Scand*. 66 103 1977.
14. Jorgensen P L. The role of aldosterone in the regulation of $(Na^+ + K^+)$ ATPase in rat kidney. *J Steroid Biochem*. 3 181 1977.
15. Krans M, Krecsek I & Popp M. The development of aldosterone production by the adrenal glands of normally and prematurely weaned rats. *Biol Neonat*. 11 338 1967.
16. Kowarski A, Katz H & Migeon C J. Plasma aldosterone concentration in normal subjects from infancy to adulthood. *J Clin Endocrinol Metab*. 38 489 1974.
17. Merlet-Benschou C & de Rouffignac D. Renal clearance studies in fetal and young guinea pigs: effect of salt loading. *Am J Physiol*. 232 178 1977.
18. Renner E. *Milch und Milchprodukte in der Ernährung des Menschen*. Volkswirtschaftliches Verlag GmbH Kempfen—Verlag Th. Mann OHG Hildesheim 1974.
19. Roy R N, Chance G W, Radde J C, Hill D E, Willis D M & Sheepers J. Late hyponatremia in very low birth weight infants (<1.3 kg). *Pediatr Res*. 10 536 1976.
20. Schmidt V & Horster M. Na^+ K activating ATPase activity maturation in rabbit nephron segments dissected in vitro. *Am J Physiol*. 233 F55 1977.
21. Sealey J E, Buhler F R, Laragh J H, Manning E L & Brunner H R. Aldosterone excretion. *Circ Res*. 31 367 1977.
22. Siegel S M, Fisher D A & Oh W. Serum aldosterone concentrations related to sodium balance in the newborn infant. *Pediatrics*. 41 410.

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GROUP B STREPTOCOCCAL COLONIZATION OF PREGNANT WOMEN AND THEIR NEONATES

Epidemiological Study and Controlled Trial of Prophylactic Treatment of the Newborn

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ABSTRACT Gerard P Verghote-D'Hulst M Bachy A and Duhaut G (Department of Neonatal Medicine Department of Obstetrics and Laboratory of Microbiology Clinique Notre Dame Charleroi Belgium) Group B streptococcal colonization of pregnant women and their neonates: epidemiological study and controlled trial of prophylactic treatment of the newborn. *Acta Paediatr Scand* 68 819 1979.—Colonization with group B streptococci of the genital tract was studied in 1115 women during the last trimester of pregnancy. 76 or 6.8% were found to harbour this bacterium. The incidence of contamination was significantly higher among Belgian women than among parturients of Mediterranean origin ($p < 0.001$). It was also more frequent in primigravidae ($p < 0.05$) and in the poorer ($0.10 < p < 0.05$). At the time of admission in the delivery room it was noticed that rupture of the amniotic membranes for more than 24 hours was more often associated with group B streptococcal carriage by the mother ($p < 0.001$). 29 out of 68 (42.6%) infants born to group B streptococci positive mothers were colonized at birth. 67 of them were submitted to a controlled trial of immediate versus delayed penicillin therapy. 44.8% and 42.1% of the neonates were contaminated at birth in each group of treatment respectively. No instance of group B streptococcal infection developed in either group. This suggests that immediate therapy with penicillin of infants of group B streptococci positive mothers has no definite advantage upon delayed treatment.

KEY WORDS Group B streptococcus pregnancy epidemiology antibiotic prophylaxis

Early onset group B streptococcal (GBS) infection represents an important cause of mortality in the neonatal period (1, 10, 11, 17, 19). Epidemiological studies have shown that contamination of the neonate is secondary to the presence of GBS in the maternal genital tract (3, 11, 15). Some 2-25% of pregnant women carry this bacterium during the last trimester of pregnancy (2, 8, 7, 11-13, 15, 16). Some 15-70% of their neonates are found contaminated immediately after birth (2, 6, 11, 13) but only very few of these infants will develop a severe systemic infection (2); the mortality of which is close to 60% (1, 10, 11, 17, 19).

Therapeutic trials aiming at prevention of infection in the newborn by eradication of

GBS from the maternal organism have been conducted with inconclusive results (11, 12, 13).

Various approaches to the treatment of infants born to GBS positive mothers have been suggested. Steigman (18) raises the question whether a single systematic injection of penicillin G to every neonate after birth could prevent the development of the disease. Because early GBS infection often manifests itself through respiratory symptoms, Cochran (9) advises penicillin administration to all infants presenting respiratory distress until results of cultures are obtained. Ablow et al (1) are in favour of early treatment of newborns presenting factors of risk: infants born

Table 1 Correlations between maternal GBS colonization and some factors or events

Factors	No of parturients colonized with GBS	r	P
Origin			
Belgian	62/576	10.76	<0.001
Mediterranean	14/538	2.60	
Gestational status			
Primigravidae	17/414	8.94	<0.05
Plurigravidae	39/701	5.56	
Maternal age			
Less than 20	11/193	6.74	N.S.*
21-30	57/790	7.21	
31-35	4/93	4.30	
More than 35	2/19	5.13	
Economic status			
I	25/487	5.13	N.S.
II	8/94	8.51	
III	13/176	7.39	
IV	7/77	9.09	
V	7/15	13.33	
Duration of rupture of membranes			
Less than 24 hours	70/1090	6.42	<0.001
More than 24 hours	6/25	24.00	

* χ^2 square test

* Non significant

to GBS positive mothers: prematurity, prolonged rupture of the amniotic membranes, early septic syndrome.

In 1976 two infants born in our Maternity developed a GBS sepsis and one of them died. This prompted the present study. As the epidemiology of GBS colonization during pregnancy in populations living in Belgium is unknown, it was decided to obtain data for the area of Belgium served by our maternity unit. Simultaneously we tested the hypothesis whether immediate antimicrobial therapy of newborns of GBS positive mothers could prevent the development of a systemic infection.

MATERIAL AND METHODS

Screening during the third trimester of pregnancy

Commencing January 4 1977 all pregnant women attending the antenatal clinic of our maternity unit had a vaginal culture performed around the 32nd-34th week. Two

obstetricians included their private parturients in the study. Only data concerning women who delivered before the end of January 1978 were computed. The maternity unit has an annual delivery rate of about 1700. Half this population is Belgian and the other half are women of Mediterranean origin, many of them being recent immigrants.

Screening in the neonate

Immediately after birth bacteriological samples were taken from infants born to known GBS positive mothers. Cultures were taken from the external auditory canal, the gastric aspirate and the fetal side of the placenta.

Bacteriological procedures

Whenever feasible the bacteriological samples were processed immediately. Otherwise they were kept in Stuart transport medium. The swabs or the gastric fluid were spread on 5% agar blood plates under an atmosphere supplemented with CO₂ and inoculated in enriched broth. Identification of the organisms was made by direct examination. Gram staining, study of the hemolytic character of the colonies, resistance to bacitracin and agglutination with commercially available anti sera (Phadebas Brocides®). No further subtyping was performed.

Controlled study of penicillin therapy of the neonate

Infants born to mothers who had been found GBS carriers were considered for this study. Babies born on an odd day received penicillin G in a dose of 50 000 to 100 000 Units/kg/day intra-muscularly in two equal doses from the delivery room on and after collection of the bacteriological samples had been completed. This treatment was continued until the results of the cultures were known. If one site was positive for GBS the antibiotic was given until the age of 7 days. If the three samples were negative then the treatment was discontinued. This group is called immediate treatment. Therapy of infants born on an even day was delayed until reception of the results of the cultures. They received penicillin only if at least one sample was positive for GBS. This group is referred as late treatment.

RESULTS

Prenatal screening

1115 women had a vaginal culture performed during the last trimester of pregnancy. 76 of them (6.82%) carried GBS in the genital tract. Correlations between maternal GBS colonization and some factors or events were analysed (Table 1).

Origin 62 out of 576 Belgian women (10.7%) were found to carry GBS whereas 14 out of 538 women of Mediterranean origin (2.6%) were positive, a difference which is highly significant ($p < 0.001$, χ^2 square test).

Number of pregnancies 37 out of 414 women (8.9%) who were pregnant for the first time were GBS positive versus 39 out of 701 (5.6%) plurigravidae ($p < 0.05$)

Maternal age Among 193 women aged less than 20 13 (6.74%) were GBS positive 57 out of 790 (7.21%) 21–30-year old pregnant women were GBS carriers 4 out of 93 (4.3%) were positive in the 31–35 age group and 2 out of 39 (5.13%) women aged more than 35. Although the incidence of GBS colonization falls after the age of 30 the difference does not reach statistical significance.

Economic status This was determined on the basis of the husband's profession. Five classes were constituted: classes I and II were of lower income; classes III and IV were middle classes and class V had the higher income. The incidence of GBS contamination was the lowest in class I (25/487 or 5.13%) similar in classes II to IV (respectively 8/94 or 8.51% for class II 13/176 or 7.39% for class III and 7/77 or 9.09% for class IV) highest in class V 2/15 or 13.33%. A difference of borderline statistical significance (0.10 < $p < 0.05$) was found between class I and all the others.

At time of admission to the delivery room a correlation was established between the duration of rupture of the amniotic membranes and presence of GBS. In 1090 women the membranes were broken for less than 24 hours 70 of them (6.4%) were GBS carriers. Among 25 cases of rupture of the membranes for more than 24 hours 6 women (24%) were known GBS carriers. This difference is highly significant ($p < 0.001$).

Contamination of the neonate at birth

68 infants born to the 76 last trimester GBS positive mothers could be studied. 29 of them (43.6%) had at least one site positive for GBS. A trend to an increase of preterm delivery or low birth weight was observed among infants born to GBS positive mothers 11/107 (10.3%) preterm infants were born of contaminated mothers compared with 65/1008 (6.44%) in

infants born after 37 weeks 5/43 (11.62%) babies who weighed less than 2500 g at birth were from GBS positive mothers as opposed to 71/1072 (6.62%) infants of more than 2500 g. However those differences do not reach statistical significance.

Controlled study of penicillin therapy of the neonate

Among the 68 infants born to GBS positive mothers one was a stillborn who was found negative for GBS. This left 67 babies for the trial. 29 were assigned to the immediate treatment group 13 of them (44.8%) were subsequently found colonized with GBS. They completed the seven day course of penicillin. All of them remained free of infectious symptoms. Among the 38 infants of the late treatment group 16 (42.1%) were contaminated and received penicillin once the results of the cultures were known usually at an age of 24 to 48 hours. No baby in this group had clinical signs that could be related to GBS infection.

No case of systemic GBS disease was observed during the period of the study as compared with two GBS sepsis—one fatal—in 1976.

To our knowledge no instance of late GBS meningitis was observed in any patient of either treatment group. No secondary ill effect could apparently be attributed to the antibiotic therapy.

DISCUSSION

The incidence of 6.8% of colonization with GBS during the last trimester of pregnancy in the population we surveyed is lower than the rate reported by Scandinavian authors 18% in Norway (7) and 14% in Sweden (6). It is close to most data reported from several centres in the USA (2, 3, 11, 15) where however regional variations as wide as 2 and 25% were evidenced.

The population attending our Maternity is heterogeneous as far as its ethnic origin is concerned. It was therefore interesting to note

Table 1 Correlations between maternal GBS colonization and some factors or events

Factors	No of parturients colonized with GBS	%	P
Origin			
Belgian	62/576	10.76	<0.001
Mediterranean	14/538	2.60	
Gestational status			
Primigravidae	37/414	8.94	<0.05
Plurigravidae	39/701	5.56	
Maternal age			
Less than 20	13/193	6.74	N.S.*
21-30	57/790	7.21	
31-35	4/93	4.30	
More than 35	2/39	5.13	
Economic status			
I	25/487	5.13	N.S.
II	8/94	8.51	
III	13/176	7.39	
IV	7/77	9.09	
V	2/15	13.33	
Duration of rupture of membranes			
Less than 24 hours	70/1090	6.42	<0.001
More than 24 hours	6/25	24.00	

* χ^2 square test

* Non significant

to GBS positive mothers, prematurity, prolonged rupture of the amniotic membranes, early apnoeic syndrome.

In 1976 two infants born in our Maternity developed a GBS sepsis and one of them died. This prompted the present study. As the epidemiology of GBS contamination during pregnancy in populations living in Belgium is unknown, it was decided to obtain data for the area of Belgium served by our maternity unit. Simultaneously we tested the hypothesis whether immediate antimicrobial therapy of newborns of GBS positive mothers could prevent the development of a systemic infection.

MATERIAL AND METHODS

Screening during the third trimester of pregnancy

Commencing January 4 1977 all pregnant women attending the antenatal clinic of our maternity unit had a vaginal culture performed around the 32nd-34th week. Two

obstetricians included their private parturients in the study. Only data concerning women who delivered before the end of January 1978 were computed. The maternity unit is an annual delivery rate of about 1,000. Half this population is Belgian and the other half are women of Mediterranean origin, many of them being recent immigrants.

Screening in the neonate

Immediately after birth bacteriological samples were taken from infants born to known GBS positive mothers. Cultures were taken from the external auditory canal, the gastric aspirate and the fetal side of the placenta.

Bacteriological procedures

Whenever feasible the bacteriological samples were processed immediately. Otherwise they were kept Stuart transport medium. The swabs or the gastric fluid were spread on 5% agar blood plates under an atmosphere supplemented with CO₂ and inoculated in enrichment broth. Identification of the organisms was made by direct examination, Gram staining, study of the hemolytic character of the colonies, resistance to bacitracin and agglutination with commercially available anti sera (Phadeb Brocades®). No further subtyping was performed.

Controlled study of penicillin therapy of the neonate

Infants born to mothers who had been found GBS carriers were considered for this study. Babies born on an odd day received penicillin G in a dose of 40,000 to 100,000 U/kg/day intramuscularly in two equal doses from delivery room on and after collection of the bacteriological samples had been completed. This treatment was continued until the results of the cultures were known. If the site was positive for GBS the antibiotic was given until the age of 7 days. If the three samples were negative the treatment was discontinued. This group is called immediate treatment. Therapy of infants born on an even day was delayed until reception of the results of the cultures. They received penicillin only if at least one sample was positive for GBS. This group is referred as late treatment.

RESULTS

Prenatal screening

1115 women had a vaginal culture performed during the 1st trimester of pregnancy. 76 of them (6.82%) carried GBS in the genital tract. Correlations between maternal GBS colonization and some factors or events were analysed (Table 1).

Origin 62 out of 576 Belgian women (10.7%) were found to carry GBS, whereas 14 out of 538 women of Mediterranean origin (2.6%) were positive, a difference which is highly significant ($p < 0.001$, χ^2 square test).

REFERENCES

- 1 Ablow H C Driscoll S G Effman E L Gross I Jolles C J Uauy R & Warshaw J B A comparison of early onset streptococcal neonatal infection and the respiratory distress syndrome of the newborn *N Engl J Med* 294 65 1976
- 2 Baker C J & Barrett F F Transmission of group B streptococci among parturient women and their neonates *J Pediatr* 81 919 1973
- 3 Baker C J Barrett F F & Yow M D The influence of advancing gestation on group B streptococcal colonization in pregnant women *Am J Obstet Gynecol* 122 870 1975
- 4 Baker C J & Kasper M L Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection *N Engl J Med* 294 753 1976
- 5 Baker C J Goroff D K Alpert S Crockett V A Zinner S H Evrard J R Rosner H & McCormack W M Vaginal colonization with group B streptococcus: A study in college women *J Infect Dis* 135 39 1977
- 6 Bergqvist G Hurvell B Thal E & Vaclovinkova V Neonatal infections caused by group B streptococci *Scand J Infect Dis* 3 709 1971
- 7 Bevanger L Carrier rate of group B streptococci with relevance to neonatal infections *Infection* 2 173 1974
- 8 Bobitt J M The group B beta hemolytic streptococcus *Semin Perinatol* 1 51 1977
- 9 Cochran W M Hyaline membrane disease and infection in the newborn *N Engl J Med* 294 844 1976
- 10 Eickhoff T C Klein J D Daly K Ingali D & Finland M Neonatal sepsis and other infections due to group B beta hemolytic streptococcus *N Engl J Med* 271 171 1964
- 11 Franciosi R A Knostman J D & Zimmerman R A Group B streptococcal neonatal and infant infections *J Pediatr* 87 87 1973
- 12 Gordon J S & Sbarra A J Identification technique of isolation and treatment of group B streptococci in obstetric patients *Am J Obstet Gynecol* 126 1073 1976
- 13 Hall H Barnes W Sumathy V Rhodes P & Favez J Antibiotic treatment of parturient women colonized with group B streptococci *J Pediatr* 86 975 1975
- 14 Hammerschlag M R Baker C J Alpert S Kasper D L Rosner I Thurston P Webb B J & McCormack W M Colonization with group B streptococci in girls under 16 years of age *Pediatrics* 60 473 1977
- 15 Hood M Janney A & Dameron G Beta hemolytic streptococcus group B associated with problems in the perinatal period *Am J Obstet Gynecol* 82 809 1961
- 16 Horn K A Zimmerman R A Knostman J D & Meyer W T Neurological sequelae of group B streptococcal infection *Pediatrics* 53 901 1974
- 17 Quirante J Ceballos R & Cassidy G Group B beta hemolytic streptococcal infection in the newborn *Am J Dis Child* 128 659 1974
- 18 Steigman A J Bottone E J & Hanna M A Does intramuscular penicillin at delivery prevent group B beta hemolytic streptococcal disease of the newborn infant? *J Pediatr* 87 496 1975
- 19 Vollman J H Smith W L Ballard E T & Light I J Early onset group B streptococcal disease: clinical roentgenographic and pathologic features *J Pediatr* 89 199 1976

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that the incidence of GBS colonization was significantly higher among Belgian women (10.8%) than in women of Mediterranean origin (2.7%).

A significantly higher incidence was also found in primigravidae women than in plurigravidae. Pregnant women from families with a lower economic income had a tendency to be less often contaminated. Maternal age did not seem to play a role.

It is worth stressing that the various factors associated with a lower rate of colonization with GBS were often present together: repeated gestation and low economic status were more frequent in the Mediterranean population.

Factors that could influence the differences in the rate of GBS carriage during pregnancy among the subgroups we studied are not well known. Ethnic or genetic influences are suggested by our findings, although other authors (3, 5) did not demonstrate such differences. Immunological factors such as ability to synthesize antibodies against GBS must also be considered (4, 14). Genital carriage of GBS by male sexual partners is another factor worth investigating (11).

In the present study we found a correlation between prolonged rupture of the amniotic membranes and previous presence of GBS in the cervicovaginal flora. These results are in accordance with Miller et al. (3). They suggest that presence of GBS could favour premature rupture of the membranes.

Not every infant born to a contaminated mother necessarily acquires this organism during the process of labour and birth. In our series 29 out of 68 (42.6%) were colonized. In other studies incidences of 71% (2), 23.5% (6), 21.5% (13) and 17.6% (11) were reported. Factors that possibly protect the newborn against contamination by GBS are unknown.

The aim of the study of antimicrobial treatment of infants born to GBS positive mothers was to test the hypothesis that immediate therapy could prevent the development of severe early pneumonic septic forms of GBS

disease. When designing the study, it was estimated that the observation of a single case of severe infection in the delayed treatment group would represent an argument in favour of immediate penicillin therapy of these infants. Conversely, the appearance of a septic state in a baby assigned to early treatment would imply that even immediate treatment would be of limited value in prophylaxis of the disease. A statement often suggested by the fulminating aspect of this infection in some babies. As no instance of GBS infection was observed in either group, these questions could not be answered.

Early treatment has several possible inconveniences. It was sometimes difficult for the parents to understand the treatment of an apparently healthy infant. As about 60% of the babies born to GBS positive mothers were not contaminated at birth, systematic treatment would result in unnecessary administration of penicillin to a large number. Penicillin therapy had no apparent untoward effect but could carry a potential risk of subsequent sensitization. Moreover, the physiological microbial colonization of the neonate could be modified by this treatment.

Other means of prevention of GBS disease in the newborn are still to be explored. Possible means include the study of protective effects of maternal specific antibodies against some types of GBS (4, 14), new controlled trials of antimicrobial treatment of pregnant women, quantitative estimations of the colonization of the maternal genital tract or invasion of the amniotic cavity at time of incipient labour (8).

Despite the low septic attack rate in neonates but because of the high mortality related to GBS infection, it seems useful to screen pregnant women for presence of this bacterium and to consider their infants as being in great risk of infection if abnormal perinatal events such as prematurity, prolonged rupture of the amniotic membranes, maternal pyrexia, birth asphyxia and early respiratory distress are present.

REFERENCES

- 1 Ablow R C Driscoll S G Effman E L Gross I Jolles C J Uauy R & Warshaw J B A comparison of early-onset streptococcal neonatal infection and the respiratory-distress syndrome of the newborn *N Engl J Med* 294 65 1976
- 2 Baker C J & Barrett F F Transmission of group B streptococci among parturient women and their neonates *J Pediatr* 83 919 1973
- 3 Baker C J Barrett F F & Yow M B The influence of advancing gestation on group B streptococcal colonization in pregnant women *Am J Obstet Gynecol* 127 870 1975
- 4 Baker C J & Kasper D L Correlation of maternal antibody deficiency with susceptibility to neonatal group B streptococcal infection *N Engl J Med* 294 753 1976
- 5 Baker C J Goroff D K Alpert S Crockett A Zinner S H Evrard J R Rosner B & McCormack W M Vaginal colonization with group B streptococcus: A study in college women *J Infect Dis* 135 39 1977
- 6 Bergqvist G Hurvell B Thal E & Vaclavinkova V Neonatal infections caused by group B streptococci *Scand J Infect Dis* 3 09 1971
- 7 Bevanger L Carrier rate of group B streptococci with relevance to neonatal infections *Infection* 2 173 1974
- 8 Bobitt J R The group B beta hemolytic streptococcus *Semin Perinatal* 1 51 1977
- 9 Cochran W B Hyaline membrane disease and infection in the newborn *N Engl J Med* 294 844 1976
- 10 Eickhoff T C Klein J D Daly J Ingall D & Finland M Neonatal sepsis and other infections due to group B beta hemolytic streptococci *N Engl J Med* 271 171 1964
- 11 Franciosi R A Knostman J D & Zimmerman M A Group B streptococcal neonatal and infant infections *J Pediatr* 87 707 1973
- 12 Gordon J S & Sbarra A J Incidence technique of isolation and treatment of group B streptococci in obstetric patients *Am J Obstet Gynecol* 176 1073 1976
- 13 Hall R Barnes W Sumathy V Rhodes P & Faye J Antibiotic treatment of parturient women colonized with group B streptococci *J Pediatr* 86 975 1975
- 14 Hammerschlag M R Baker C J Alpert S Kasper D L Rosner I Thurston P Webb B J & McCormack W M Colonization with group B streptococci in girls under 16 years of age *Pediatrics* 60 473 1977
- 15 Hood M Janney A & Dameron G Beta hemolytic streptococcus group B associated with problems in the perinatal period *Am J Obstet Gynecol* 87 809 1961
- 16 Horn K A Zimmerman R A Knostman J D & Meyer W T Neurological sequelae of group B streptococcal infection *Pediatrics* 53 501 1974
- 17 Quirante J Ceballos R & Cassidy G Group B beta hemolytic streptococcal infection in the newborn *Am J Dis Child* 128 659 1974
- 18 Steigman A J Bottone E J & Hanna B A Does intramuscular penicillin at delivery prevent group B beta hemolytic streptococcal disease of the newborn infant? *J Pediatr* 87 496 1975
- 19 Vollman J H Smith W L Ballard E T & Light I J Early onset group B streptococcal disease: clinical roentgenographic and pathologic features *J Pediatr* 89 199 1976

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C REACTIVE PROTEIN (CRP) IN EARLY DIAGNOSIS OF NEONATAL SEPTICEMIA

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ABSTRACT Sabel K. G. and Wadsworth Ch. (Department of Paediatrics and Department of Clinical Immunology, University of Göteborg, Sweden). C reactive protein (CRP) in early diagnosis of neonatal septicemia. *Acta Paediatr Scand* 68 825 1979. The usefulness of CRP in early detection of neonatal septicemia/meningitis and urinary tract infection was studied in a neonatal unit using a semiquantitative latex agglutination as a rapid screening method and electroimmuno assay as reference method for CRP determination. In 94% of non infected infants CRP was ≤ 15 mg/l and 87% had CRP < 10 mg/l up to 3 days of age. After 3 days of age 96% had CRP < 10 mg/l. The initial CRP level was increased in 16 out of 18 patients (89%) with bacterial septicemia. Low CRP was seen in one patient with total agranulocytosis and septicemia from *Streptococcus* type B and in one patient with *Staphylococcus albus* sepsis. A rise in CRP was also seen in very pre term infants with septicemia. Increased initial CRP was uncommon in neonatal urinary tract infection (2 of 9) but a rise was seen in 3 additional patients. A comparison between CRP, total neutrophil blood cell count and band neutrophil count as diagnostic parameters was in favour of CRP at this early stage of infection. CRP is of definite value as an aid in early diagnosis of neonatal septicemia and bacterial meningitis.

KEY WORDS C reactive protein, neutrophils, septicemia, meningitis, urinary tract infection, neonates.

Septicemia of the neonate is usually a life threatening disease where early diagnosis and prompt treatment are essential for the outcome. The early signs and symptoms of infection are often unspecific and vague. Therefore routine laboratory aids which are simple, rapid and specific are badly needed. The usefulness of blood parameters such as neutrophil blood cell count (2, 3) and band neutrophil count (1) have been reported. C reactive protein (CRP) which is present in only small amounts in serum of healthy individuals (5) is involved in several processes of the unspecific immunologic defence (e.g. 7, 8). In severe infections or inflammatory reactions a striking rise in the serum concentration is often seen. Sabel & Hanson (10) have recently shown that during the first five days after diagnosis of septicemia-meningitis in infants increased CRP levels were found in at least 85% of the patients. In several cases they observed high

CRP values during the first few hours after clinical symptoms had appeared. This suggested the possibility that the rise of CRP was sufficiently rapid and specific to serve as a definitive aid in the early diagnosis of septicemia.

The present report studies the efficacy of CRP for the early diagnosis of neonatal infections of pre term and term babies and presents a comparison of CRP with total neutrophil and band cell counts in this respect.

MATERIAL AND METHODS

A prospective investigation was performed from August 1973 to July 1975. The study included infants up to 30 days of age with suspect general infection at an early stage either because of clinical signs and symptoms or because of chorio-amnionitis or infection of the mother.

Of the original 165 infants in the prospective study 36 (22%) had to be excluded because they did not fulfil the criteria (samples for cultivation and CRP taken on different days, insufficient samples or samples taken after

Table 1 Infants with verified septicemia or meningitis Group Ia

CRP=C reactive protein
CSF=Cerebrospinal fluid

Patient no	Age at sampling (days)	CRP (mg/l)	Total neutrophil (count/mm ³)	Total band (count/mm ³)	Cultured agent	
					Source	Designation
1	5/24	6	0	0	Blood	<i>β-Streptococcus</i> group B
	17/24	60	300	140	Blood CSF	<i>β-Streptococcus</i> group B
	18	200	1700	2100		
3	17/24	6			Blood	<i>β-Streptococcus</i> group B
	7	34				
4	<1	70			Blood CSF	<i>β-Streptococcus</i> group B
	1	70				
5	18	200			Blood	<i>β-Streptococcus</i> group B
	5	33				
6	3	65	14200	600	Blood	<i>β-Streptococcus</i> group B
	6	105				
7	16	9			Blood	<i>β-Streptococcus</i> group B
	17	20				
8		23			Blood	<i>Streptococcus</i>
	3	130				
9	4	1	3400	140	Blood	<i>Staphylococcus albus</i>
10	4	40			Blood	<i>Staphylococcus albus</i>
11	7	23	4700	60	Blood	<i>Staphylococcus aureus</i>
	8	2	2500	90		
12	1/24	24			CSF lungs urine	<i>Pneumococcus</i> <i>Escherichia coli</i>
13	4	25	3000	700	Blood CSF	<i>Escherichia coli</i>
	7	30				
14	4	0	1400	240	Blood	<i>Escherichia coli</i> + <i>enterococcus</i>
	9	15				
15	5	25	400	930	Blood	<i>Escherichia coli</i>
16	7	30			Blood	<i>Escherichia coli</i>
	8	140				
17	18	60			Blood	<i>Escherichia coli</i>
	3	0				
18	3	100			Blood	<i>Pseudomonas aeruginosa</i>
19	4	14			CSF	Coxsackie B virus
	6	15	11400	90		
20	5	6			CSF	ECHO virus type 9
	6					

Died

116 mg/l at 0-3 days of age and 8 mg/l at 4-30 days of age for non infected infants

CRP in infants with septicemia or meningitis (Group Ia)

This group consisted of 20 infants with verified septicemia or meningitis. As is seen in Fig 2 and Table 1, 16 out of 18 patients (89%) with

bacterial septicemia or meningitis had increased CRP levels in the initial CRP sample. Case no. 1 with a fatal congenital infection caused by *β-Streptococcus* group B had a CRP within the normal range but only one sample was obtained at 5 h of life. This patient also had total agranulocytosis. One patient (no. 9) with *Staph. albus* sepsis also had a low

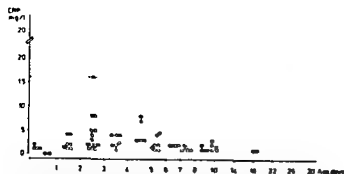


Fig. 1 Group III non infected infants ($n=98$). Concentration of CRP in first sample related to age of the infant. Normal limit is indicated.

antibiotics had been given). From the remaining 129 patients 232 samples were taken for CRP determinations. In addition all infants born at the hospital during August 1975 to July 1976 with a diagnosis of septicemia, meningitis or urinary tract infection (UTI) were retrospectively studied (55 samples from 12 patients).

Depending on the clinical course and the results of the cultivations the 141 infants were divided into four groups.

Group Ia: 20 infected infants with septicemia or meningitis verified by positive cultures from the blood in the 18 of bacterial origin. The other 2 infants had positive viral cultures from the cerebrospinal fluid (CSF) (see Table 1). Four babies had bacterial meningitis with positive cultures from the CSF.

Group Ib: 9 infected infants with urinary tract infection verified by cultivation of *Escherichia coli* (>1000 organisms/ml) in urine obtained by bladder puncture but other cultivations were negative (see Table 2).

Group II: 14 suspect infected infants where cultivations of blood, CSF and bladder urine were negative. These patients like those in Groups Ia and b had a clinical course suggesting general infection.

Group III: 98 non infected infants who satisfied the aforementioned criteria for care in the Neonatal Unit but where the cultivations were negative. Furthermore the subsequent clinical course was not suggestive of infection and no antibiotic treatment was given.

Sampling techniques: Urine was obtained by supra pubic bladder puncture and CSF by lumbar puncture. Blood for aerobic and anaerobic cultivation was taken from peripheral veins. In about one half of the cases capillary blood from free flowing heel pricks was used for the total and differential white cell counts.

CRP determinations were performed with two methods on serum from capillary, venous or arterial blood, often as small sample as 0.5 ml of blood.

Latex agglutination was performed mainly according to the manufacturer's instructions (AG Behringwerke, Marburg, Germany) using 10 μ l aliquots of reagents and patient's serum undiluted and diluted 1/5 and 1/20 with Veronal buffer 75 mmol/l, pH 8.2 with 12.3 mmol Ca lactate/l and 6 mmol Na₂SO₄/l. After 5 min reaction time the degree of agglutination was read at 12.5 \times magnification and registered as negative \pm 1+ or 2+. An arbitrary scale was established for each batch of latex particles

using sera with known CRP content as determined by electroimmuno assay. Thus a preliminary semiquantitative report of CRP level is negative <10 , 10–40, 40–50, >50 mg/l could be received within 1 h of sampling.

The electroimmuno assay (EIA) originally presented by Laurell (6) was employed for more exact quantitation as described by Wadsworth (11). Plates were run 3 times per week with results reported the day after each run.

Statistical methods employed were a non parametric tolerance interval (9) and the Wilcoxon rank sum test.

RESULTS

CRP in non infected infants (Group III)

The initial CRP values determined with EIA in the 98 non infected infants during the first month of life are shown in Fig. 1. The initial sample was obtained at 0–3 days of age in 4, at 4–7 days in 25 and at 8–30 days in 22 patients. CRP values were squewly distributed when values for each day were considered. 0–3 days of age 94% had ≤ 15 mg/l and 80% <10 mg/l. At 4–7 days of age 96% had less than 10 mg/l. Using the non parametric tolerance interval (95–50 TI) the upper limit was 5

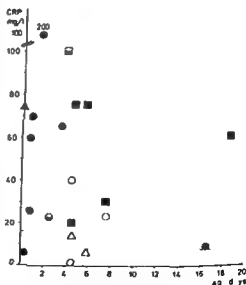


Fig. 2 Group Ia infants with verified septicemia/meningitis ($n=20$). Concentration of CRP in first sample related to age. Normal limit is indicated. Symbols: \bullet *Streptococcus* group III, \circ *Staph aureus*, \square *Staph albus*, \triangle *Pneumococcus* + *E. coli*, \blacktriangle *Pseudomonas aeruginosa*, \blacksquare *Corysacke B* or *EHEC meningitis*.

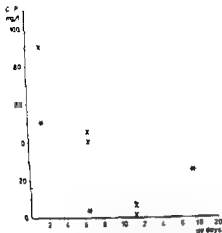


Fig. 4 Group II infants with suspected infection not verified by culture. Concentration of CRP in first sample related to age. Normal limit is indicated. Infants not treated by antibiotics marked by x.

tation produced moderate to high amounts of CRP. When comparing infected pre term babies (27–36 weeks $n=11$) with full term newborns with verified bacterial sepsis/meningitis ($n=7$) no significant difference was found in CRP levels between the groups.

CRP versus total neutrophil blood cell count

The results of total neutrophil counts in 71 patients at the time of initial CRP sample was compared with the data presented by Gregory & Hey (2). In the present study an early total neutrophil count fell within the region within which 90% of all normal neutrophil counts are found in 4 out of 8 tested cases of bacterial septicemia/meningitis, in all 3 tested cases with UTI and in 5 of the 10 tested cases in Group II. Furthermore 20% of the non infected tested babies (Group III) were false positive.

CRP versus band neutrophil count

The distribution of early samples of nonsegmented (band) polymorphonuclear leucocytes was investigated in 50 babies during the first 5 days of life. With one exception they were

all within the 95% range for normal full term infants reported by Akenzua et al (1). Thus 7 infants with bacterial septicemia/meningitis and one case of UTI were rated false negative while 7 out of the 8 tested patients in the suspect infected group were within the normal range.

Utility of agglutination results

The agglutination results for the initial samples can be considered in relation to the patient groups. With the exception of Group 1b the distribution seems reasonable. 85% of the non infected infants had negative \pm or + agglutination results while the infected 1a and suspect infected Group II had 10 or 15% with in this range. Agglutination findings were initially low in 7 out of 9 infants with verified bacteremia. It should be noted however that of the 23 sera with initial 2+ agglutination which were at first reported as 10–30 mg/l 12 were from infants later designated as non infected. Nine of these proved to have EIA ratings in the normal range for age.

Some of these 2+ (10–30 mg/l) reports can be followed up in Table 3 where the detailed results are given for second samples with a higher CRP level than that found in the first. In most instances the infected infants responded in the next sample taken within 1 or 2 days with high levels of CRP in contrast to the non infected.

DISCUSSION

The need is obvious for a quick and safe guide to direct the difficult decision of treatment or no treatment of a neonate with vague symptoms which could be due to sepsis. It is of greatest importance for the outcome to catch the earliest possible deviation from normal in any laboratory parameter used for this purpose. During the last few years several authors have reported the usefulness of total neutrophil count (2, 3, 12) or total band neutrophil count (1, 12, 13) in this respect. In the present study neither total neutrophil count nor band neutrophil count were comparable to

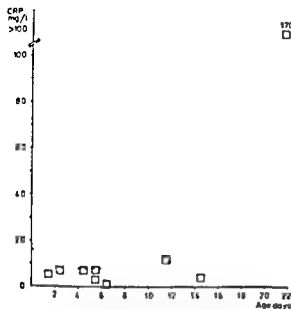


Fig. 3. Group Ib infants with verified urinary tract infection (UTI) ($n=9$). Concentration of CRP in first sample related to age. Normal limit is indicated. UTI from *E. coli* □.

CRP in his single sample. The two cases with viral meningitis had low CRP at 14 and 6 mg/l respectively. Four out of 5 cases with verified septicemia or meningitis during the first day of life already had an increased level of CRP. The earliest sample was taken at 1 hour of age (case 12).

CRP in infants with bacteremia (Group Ib)

In the group of babies with bacteremia 7 of 9 had initial CRP levels within the normal range (Fig. 3, Table 2). One patient had a slightly increased CRP of 12 mg/l and another 3 weeks old had an initial CRP of 170 mg/l. This patient (no. 29) was the only one with symptoms of UTI of several days' duration at the day of sampling and the only one with temperature over 38°C. The microsedimentation rate was 40 mm/h. In subsequent samples from three of four patients with very slight symptoms a marked increase in CRP level was observed. In 2 of the 3 patients with increasing CRP no treatment had been given for 3 days after bacteremia had been ascertained. The slight clinical symptoms remained unchanged and the temperature was 37.5°C at its highest.

Table 2. Infants with urinary tract infection verified by cultivation of *Escherichia coli* from urine taken by bladder puncture. Group Ib. CRP = C reactive protein

Patient no.	Age at sampling (days)	CRP (mg/l)
21	1	5
	2	100
	3	37
22	2	7
	5	100
23	4	7
24	5	7
25	5	3
	9	1
26	6	1
	11	17
27	23	3
	14	4
	17	60
	20	6
29	21	170
	24	80
	25	5
	28	5

After treatment had been initiated

CRP in suspected infected infants (Group II)

Ten of the 14 cases of suspected infection not verified with positive culture had high levels of CRP: one was slightly increased and 3 had CRP within the normal range (one with aseptic meningitis, one with slight aspiration and one with small infiltrates on X-ray suggestive of pneumonia) (Fig. 4). Seven of the 14 cases had pulmonary infiltrates suggestive of pneumonia or aspirations with secondary pneumonia. Two cases with initial CRP 40 and 90 mg/l respectively had symptoms highly suggestive of septicemia but cultures were negative.

CRP in relation to gestational age

The initial CRP values taken during the first week of life were grouped according to the gestational age of the infants. Four out of 5 infants with septicemia after 27–32 weeks of ges-

there is about 15% false negatives in the present series. With more experience with the SIA method this figure may be reduced. In a newborn with vague symptoms which may or may not indicate bacterial infection CRP as well as the clinical condition should be followed. If the clinical condition is satisfactory and the CRP not rising above normal limits antibiotics usually can be withheld. In our experience the use of CRP as a diagnostic aid has reduced administration of antibiotics in the nursery.

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REFERENCES

- Akenzua G I, Hui Y T, Milner R & Zipursky A. Neutrophil and band counts in the diagnosis of neonatal infections. *Pediatrics* 54: 38, 1974.
- Gregory J & Hey E. Blood neutrophil response to bacterial infection in the first month of life. *Arch Dis Child* 47: 747, 1972.
- Haider S A. Polymorphonuclear leucocyte count in diagnosis of infection in the newborn. *Arch Dis Child* 47: 394, 1972.
- Jodal U, Lindberg U & Lincoln K. Level of diagnosis of symptomatic urinary tract infections in childhood. *Acta Paediatr Scand* 64: 701, 1975.
- Kindmark C O. The concentration of C reactive protein in sera from healthy individuals. *Scand J Clin Lab Invest* 29: 407, 1977.
- Laurell C B. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 15: 45, 1966.
- Mortensen R F, Osmond A F & Gewurz H. Effects of C reactive protein on the lymphoid system. I. Binding to thymus-dependent lymphocytes and alteration of their functions. *J Exper Med* 141: 871, 1975.
- Mortensen R F & Gewurz H. Effects of C reactive protein on the lymphoid system. II. Inhibition of mixed lymphocyte reactivity and generation of cytotoxic lymphocytes. *J Immunol* 116: 1744, 1976.
- Reed A H, Henry R J & Mason W B. Influence of statistical methods used on the resulting estimate of normal ranges. *Clinical Chemistry* 17: 775, 1971.
- Sabel K G & Hanson L Å. The clinical usefulness of C reactive protein (CRP) determinations in bacterial meningitis and septicemia in infancy. *Acta Paediatr Scand* 63: 381, 1974.
- Wadsworth Ch. A rapid spot immunoprecipitate assay method applied to quantitating C reactive protein (CRP) in pediatric sera. *Scand J Immunol* 6: 1, 63, 1977.
- Xanthou M. Leucocyte blood picture in ill newborn babies. *Arch Dis Child* 47: 741, 1972.
- Zipursky A, Palko J, Milner R & Akenzua G I. The hematology of bacterial infections in premature infants. *Pediatrics* 57: 839, 1976.

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Table 3. Consecutive samples showing a rise in C reactive protein

EIA=electroimmuno assay UTI=urinary tract infection

	At least two samples		First sample			Second sample				Interval (in days)
	n	with rise in second	Agglutination		LIA (mg/l)	Agglutination			EIA (mg/l)	
			1/1	1/5		1/1	1/5	1/10		
Gr. I a	15	7	±	-	9	+	++	±	270	1
Infected (n=20)			++	-	14	±	-	-	15	1
			++	-	23	-	++	++	130	1
			++	-	26	++	±	-	14	1
			++	±	30	N D	-	-	140	1
			++	++	60	-	++	++	700	1
			-	+	65	N D	-	-	105	3
Gr. I b	5	3	-	-	4	++	+	-	60	3
UTI (n=9)			++	-	5	+	++	-	100	1
			+	-	7	±	++	-	100	3
Gr. II	13	1	-	-	1	++	+	-	40	<1
Suspect infected (n=14)										
Gr. III	18	3	-	-	1	++	-	-	9	1
Non infected (n=94)			++	-	12	++	-	-	14	1
			++	-	14	±	-	-	15	1

CRP in the early detection of infection. Furthermore there were large numbers of false positives with total neutrophil counts and of false negatives with band neutrophil count.

In the present study on CRP we found using a modified standardized semiquantitative latex agglutination that routine results could be obtained within 2 hours. From the practical point of view a latex agglutination of - or ± can be considered negative. Between 0-3 days of age however some newborns without infection may have values of 15-20 mg/l or 1+ agglutination. The range between 10-30 mg/l that is 2+ agglutination is not as clear cut. Furthermore samples taken very early in the course of infection may be in this range. In doubtful cases it is essential to repeat the sample after a few hours since the rise of CRP seems to be very rapid.

A quantitative method which will give the result within a few hours is desirable. The electroimmuno assay as performed in our laboratory has proved to be reasonably satisfactory for quantitation although results are not available for 1-2 days and the quantitation may be low for some sera agglutinating at 2+

These challenges may be met with the rapid spot immunoprecipitate assay (SIA) (11).

Cases with neonatal UTI had initial CRP in the normal range. In some cases a sharp rise in CRP was seen. This may indicate that the infection was first confined to the bladder but later advanced to the kidneys (4).

Infection is most difficult to diagnose in very pre term infants. The capacity to form CRP is minimal and occurs quite early. CRP determination in very pre term infants may prove to be of great value in early detection of infection in these babies.

In the neonatal period a CRP rise from causes other than infection seems to be comparatively rare. In the present series approximately 5% will have false positive values with a limit set at 15 mg/l at 0-3 days and at 10 mg/l from 4 days on.

Thus a high CRP in combination with any symptoms indicating infection is highly suggestive of bacterial infection and should be treated with little risk of overtreatment. On the other hand a newborn with symptoms indicating a severe bacterial infection but with negative CRP should also be treated since

MYCOPLASMA PNEUMONIAE INFECTION

A Retrospective Review of 103 Hospitalised Children

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ABSTRACT Mok J Y Inglis J M and Simpson H (The Royal Hospital for Sick Children Edinburgh Department of Child Life and Health University of Edinburgh and Regional Virus Laboratory Edinburgh Scotland) *Mycoplasma pneumoniae* Infection—A Retrospective Review of 103 Hospitalised Children *Acta Paediatr Scand* 68 833 1979—The clinical aspects of *Mycoplasma pneumoniae* infection in 103 children under 12 years admitted to hospital over an eight year period were reviewed retrospectively. Respiratory illnesses occurred in 83 (85%) cases. The prevalence of lower respiratory tract involvement was similar in both pre-school and school children. Cough was the commonest symptom at all ages. Coryzal symptoms and wheeze were common in pre-school children. Most infants had signs of pharyngitis or otitis media. Non-specific symptoms—fever lethargy malaise anorexia and vomiting—were common accompaniments in children older than one year of age. Non-respiratory illnesses in 16 (15%) patients included gastroenteritis convulsions non-specific skin rashes and limb pain. The duration of stay in hospital ranged from two to 30 days (median five days) with apparent clinical recovery and resolution of chest X-ray abnormalities within three months in 78 (76%) patients seen for review.

KEY WORDS *Mycoplasma pneumoniae* infection hospitalised children

The spectrum of disease caused by *Mycoplasma pneumoniae* is well documented (6, 16). Reports in children have been concerned mainly with out-patients in day care centres (7) and residents in children's homes (2, 10). Infections are often mild and many children remain symptom free. There have been few reports of series of children admitted to hospital (3, 21). In a report of 44 such children during an 18 month epidemic period Stevens et al. (21) emphasised the importance of lower respiratory tract involvement and serious non-respiratory manifestations of infection.

Little attention has been paid to the effect of age on clinical presentation. We report here a retrospective hospital based study of 103 children under the age of 12 with *M. pneumoniae* infection with particular reference to the clinical features in three different age groups—under one year, one to five years and five to 12 years.

MATERIALS AND METHODS

The names of all children admitted to the Royal Hospital for Sick Children in Edinburgh with *M. pneumoniae* infection during the period from January 1968 to December 1975 were obtained from the records of the Regional Virus Laboratory in Edinburgh. Their case records were then traced and analysed. Initially laboratory investigations had been carried out on the basis of clinical suspicion of *M. pneumoniae* infection. However from 1972 onwards over 70% of patients admitted to hospital with respiratory tract infections were studied in conjunction with routine virological investigations. The series may therefore be biased to include a high proportion of patients with respiratory illnesses. No attempt has been made to assess the contribution of *M. pneumoniae* infection to the overall pattern of respiratory infections in hospital.

Throat and nasal swabs obtained from each child within a day of admission were placed in the same bottle of transport medium (3 ml Hanks balanced salt solution containing 0.7% bovine albumin, ampicillin 700 µg/ml and penicillin 150 I.U./ml) and transported to the laboratory within one hour. Before inoculation within the following three hours swabs were shaken (to liberate the cells from the cotton pledgets) and 0.75 ml added to a selective medium for *M. pneumoniae* (5). Cultures were incubated at 36°C for at least two months. Sera were tested for complement fixation antibodies to *M. pneumoniae* (4).

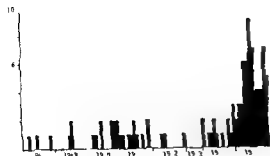


Fig 1 Distribution of admissions of children with *M. pneumoniae* infection by month and year

received two or more drugs. Of the antibiotics used, ampicillin was the most commonly prescribed (57%) followed by penicillin (34%). The remaining 9% received cotrimoxazole, sulphonamides, tetracyclines, cephalosporins or erythromycin.

Eighty-seven patients (85%) had respiratory illnesses. The main respiratory symptoms were nasal discharge, cough and wheeze, with cough being the predominant symptom at all ages. Coryzal symptoms, wheeze and vomiting were commonest under the age of five, whereas fever, malaise, lethargy, anorexia and headache were least common in this age group. The duration of symptoms prior to admission was less than one week in 47 patients (46%), one to two weeks in 24 (24%), two to four weeks in 14 (14%) and over a month in 16 (16%).

Fig 3 gives the main physical signs elicited

Table 3 White cell count (grouped) in relation to age group in 102 children with *M. pneumoniae* infection

Range	Total white count (/mm ³)	Age group (years)			Total number
		0-1	1-5	5	
Normal	4 000-10 000	6	16	27	49
High	>10 000	8	22	22	52
Low	<4 000	1			1

on examination. Signs of inflammation in the upper respiratory tract, indicative of rhinitis, pharyngitis and otitis media, affected a high proportion of patients in the first year of life. After the age of one year, crepitations in the chest was the most consistent sign of lower respiratory tract involvement. Sixteen patients (15%) had no respiratory symptoms. Their



Fig 2 Symptoms by age of 103 children hospitalised for *M. pneumoniae* infection

Table 2 Uncommon symptoms and signs in children with *M. pneumoniae* infection

Symptom/sign	No. of patients
Chest pain	7
Weight loss	7
Muscle and joint pains	7
Sinistral	6
Mucocutaneous lesions	6
Diarrhoea	4
Meningism	4
Drowsiness	4
Haemoptysis	1
Haematuria	1

Table 1. Diagnostic criteria of *M. pneumoniae* infections in 103 children

Age group (years)	Number of patients with maximum CI antibody titre to <i>M. pneumoniae</i>							Number of patients with significant changes in titre		<i>M. pneu- moniae</i> isolated
	≥160	30	640	256	512	1024	Total	Rise	Fall	
<1	1	—	—	5	—	—	6	5	—	3
1-5	—	2	1	20 (2)	1	2	26 (2)	7 (1)	1	10 (3)
>5	4	2	9	17 (3)	3	2 (1)	37 (4)	8 (2)	1	8 (6)
Total	5	4	10	42 (5)	4	4 (1)	69 (6)	20 (3)	2	21 (9) ^a

No. of patients with positive isolates in parenthesis

^a No. of patients with antibody measurements in parenthesis

Table 1 summarises the laboratory criteria for diagnosing *M. pneumoniae* infection. This was based on positive cultures for *M. pneumoniae* in 21 patients (20%), a four fold rise or fall in antibody titre in 19 (18%) and a single high titre (≥160 or ≥256) in 63 (62%). The method of reporting antibody titres was changed during the third year of the study.

It had been routine clinical practice for several years to investigate single serum samples from children with acute respiratory illnesses admitted to one of three medical wards, and to obtain second samples if symptoms had not resolved in the subsequent three to four weeks. We have therefore analysed the results obtained in single serum samples from 100 consecutive cases admitted during the first six months in 1976 (the year following an epidemic). Table 1a gives the results. Ninety-six patients had antibody titres of 64 or less.

RESULTS

Demographic

There were 62 males and 41 females—a male:female ratio of 1.5:1. Their ages ranged from 0.1 to 11.2 years (mean 5.1). Fifteen patients were less than one year old (14%), 38 between one and five years (37%) and 50 over the age of five (49%).

Fig. 1 shows the number of cases admitted each year during the study period. Most were admitted during an epidemic in 1975.

Most children had been healthy prior to the index illness. Sixteen (15%) were known to suffer from asthma. Ten (10%) had pre-existing conditions—congenital heart disease (2), Down's syndrome (2), leukaemia in remission (2), spastic diplegia (1), epilepsy (1), multiple

congenital abnormalities (1) and laryngeal papilloma (1).

Blood samples were taken during the acute phase of illness at the time of admission to hospital. The duration of illness prior to admission was similar in patients with single low antibody titres and those in whom isolates were obtained. The length of stay in hospital ranged from two to thirty days (median five days). In nine (9%) patients at least one other family member had similar symptoms.

Clinical

Fig. 2 shows the main symptoms according to age group. Fever, malaise, lethargy and anorexia were the most common general symptoms. Gastrointestinal complaints (vomiting and/or abdominal pain) were found in 42 patients (41%) while headaches and/or convulsions affected 24 (23%). Six children presented with generalised convulsions.

Sixty-five patients (63%) had been treated with antibiotics prior to admission and 15 had

Table 1a. Antibody titres to *M. pneumoniae* in single serum specimens from 100 consecutive children admitted to hospital with acute respiratory illnesses in 1976

	Titre					
	<16	16	32	64	128	256
Number	34	21	24	17	0	4

Table 4 Percentage of patients with high normal or low absolute neutrophils and lymphocyte counts derived from absolute differential white cell counts in 102 children with *M. pneumoniae* infection

Type of white cell	High	Normal	Low
Neutrophils	39	59	7
Lymphocytes	3	69	30

lated in 64 (74%) *Staphylococcus aureus* (nine patients) *Haemophilus influenzae* (8) group A beta-haemolytic streptococci (5) and pneumococci (1) were the bacteria isolated. In sputum samples from 18 patients *H. influenzae* (seven patients) group A beta-haemolytic streptococci (1) and *Pseudomonas pyocyaneus* (1) were isolated. The proportion of positive isolates from nose and throat swabs or sputum was higher in patients who had not received antibiotics. This was not significant statistically.

Cerebrospinal fluid was examined in 17 patients (16%) suspected of having meningitis. The lymphocyte count was increased in five and in two samples ECHO virus type 9 was isolated. Of two patients with an increase in neutrophils one had meningococcal meningitis. Cerebrospinal fluid was sterile in the remaining patients.

Two five year old children had co-existing infections outwith the respiratory tract. One presented with headache, vomiting and abdominal pain and was also found to have a urinary tract infection. The other who gave a two-week history of fever, anorexia and vomiting, also had malaria.

Treatment and progress

Patients with radiological evidence of pneumonia were treated with chest physiotherapy and humidified oxygen where indicated on clinical grounds. Eighty patients (78%) were treated with antibiotics. Ampicillin (40%) and penicillin (29%) were the antibiotics most

commonly prescribed. Drugs considered to be effective against *M. pneumoniae* (erythromycin or tetracycline) were first choice antibiotics in only six patients (6%). A further 25 (24%) were treated with these antibiotics when laboratory results became available.

Thirty seven patients (36%) had a temperature exceeding 38°C on admission which in most cases became normal within two days.

Following discharge 78 patients (76%) were seen for review at intervals varying from one week to three months. In 61 (78%) the chest X-ray had returned to normal a month after the initial illness. At two months only three patients had residual abnormalities which had cleared a month later.

DISCUSSION

This report describes the clinical features of *Mycoplasma pneumoniae* infection in children ill enough to require admission to hospital. The question arises whether single low antibody titres (160 or 256) are reliable (Table 1) in the diagnosis of recent *M. pneumoniae* infections. Our data does not allow a complete answer as we have not measured antibody titres in symptom free children. However many children with acute respiratory illnesses had low antibody titres in the acute phase of illness (Table 1a) even in the year following an epidemic of *M. pneumoniae* infection. In addition we are unaware of anamnestic reactions in relation to previous *M. pneumoniae* infections. It seems unlikely that significant error is introduced by imprecise criteria of diagnosis.

More than half of our patients were of pre-school age and almost one sixth less than a year old. This may seem surprising as it has been stated that pre-school children usually have mild or asymptomatic infection (7). The fact that very young or ill children are most likely to be admitted to hospital may explain the age bias in the present series.

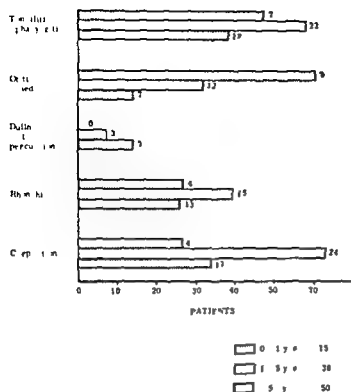


Fig. 3. Clinical signs by age of 103 children hospitalised for *M. pneumoniae* infection.

presenting complaints were gastro intestinal (7) convulsions (5) skin rashes (2) limb pruritis (1) and fever (1).

Table 2 lists uncommon symptoms and signs found in 37 of our 103 patients.

Laboratory investigations

Fig. 4 shows the proportion of children by age with radiological abnormalities. Chest X rays were abnormal in 66 of 94 patients (70%) including peribronchovascular infiltrates (48%) consolidation of one or more lobes (42%) and overinflation (10%). When present infiltrates were bilateral and diffuse (43% of the X rays showing infiltrates). Of the films with pneumonic consolidation 61% showed the lower lobes to be affected. Pleural effusion was noted in only one X ray.

Haemoglobin ranged from 9.7 to 15.2 g/dl (mean 12.5). Tables 3 and 4 show the results of total and differential white cell counts performed in 102 children in relation to normal values (1-17/18). Erythrocyte sedimentation rate (ESR, Westergren method) in 97 patients

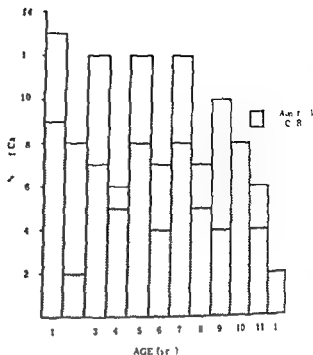


Fig. 4. Proportion of children by age with radiological abnormalities.

was normal in 30 (31%) raised in 62 (64%) and equivocal in 5 (5%). Raised ESRs ranged from 12 to 114 mm/h (mean 42).

Serum electrolyte concentrations were normal in children with a history of recurrent lower respiratory tract illnesses.

Blood films in 54 patients showed rouleaux formation in 31 (57%), atypical lymphocytes in 21 (39%) and toxic granulation of neutrophils in 19 (35%). Some patients had more than one abnormal finding on the blood film.

Viruses were isolated or viral infection inferred serologically in 19 patients. These included parainfluenza virus type 1 (four patients), parainfluenza virus type 3 (1), adenovirus type 2 (3), influenza A virus (2), Coxsackie virus B3 (2), Coxsackie virus B4 (1), ECHO virus type 1 (1), ECHO virus type 9 (2), Herpes simplex virus (2) and measles virus (1).

Bacteriological studies were carried out in nasal and throat swabs from 87 patients, 56 (64%) of whom had been treated with antibiotics. Commensal organisms only were iso-

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REFERENCES

- 1 Altman P L & Dittmer D S *Blood and other body fluids* Federation of American Societies for Experimental Biology Washington D.C. 1961
- 2 Baernstein H D, Trevisani E, Axtell S & Quiligan J J *Mycoplasma pneumoniae* (Eaton atypical pneumonia agent) in children's respiratory infections *J Pediatr* 66 8 9 1965
- 3 Bain H H, Stewart S & Simpson H Infections due to *Mycoplasma pneumoniae* in children *Arch Dis Child* 46 739 1971
- 4 Bradstreet S M P & Taylor C E D Technique of complement fixation test applicable to the diagnosis of viral disease *Monthly Bulletin of Ministry of Health and Public Health Laboratory Service* 21 96 1967
- 5 Chanock R M, Fox H H, James W D, Custer R R, White R J & Senterfit L B Epidemiology of *Mycoplasma pneumoniae* infection in military recruits *Ann NY Acad Sci* 143 484 1967
- 6 Denny F W, Clyde W A & Glezen W P *Mycoplasma pneumoniae* disease: Clinical spectrum, pathophysiology, epidemiology and control *J Infect Dis* 123 74 1971
- 7 Fernald W, Collier A M & Clyde W A Jr Respiratory infections due to *Mycoplasma pneumoniae* in infants and children *Pediatrics* 55 377 1975
- 8 Foy H M & Alexander E R *Mycoplasma pneumoniae* infections in childhood. In I Schulman (ed.) *Advances in Pediatrics* 16 301 Book Medical Publishers 1969
- 9 Foy H M, Loop J, Clarke E M, Mansy A W, Spence W F, Feigl M & Grayston J T Radiographic study of *Mycoplasma pneumoniae* pneumonia *Am Rev Resp Dis* 108 469 1973
- 10 Glezen W P, Thornburg G, Chin T Y D & Wenner H A Significance of *Mycoplasma* infections in children with respiratory disease *Pediatrics* 39 516 1967
- 11 Hers J F P & Masurel N Infections with *Mycoplasma pneumoniae* in civilians in the Netherlands *Ann NY Acad Sci* 143 447 1967
- 12 Hodges G R, Fass H G & Saslaw S Central nervous system disease associated with *Mycoplasma pneumoniae* infection *Arch Intern Med* 130 277 1970
- 13 Kjellman B Pulmonary function in children with *Mycoplasma pneumoniae* pneumonia *Infection* 4 Suppl 1 71 1976
- 14 Lerer R J & Kalavsky S M Central nervous system disease associated with *Mycoplasma pneumoniae* infection: Report of five cases and review of literature *Pediatrics* 52 658 1973
- 15 Lok J Y Q, Waugh P R & Simpson H *Mycoplasma pneumoniae* infection—A follow up study of 50 children with respiratory illnesses. Accepted for publication by *Arch Dis Child* November 1978
- 16 Murray H W, Masur H, Senterfit L B & Roberts R B The protean manifestations of *Mycoplasma pneumoniae* infection in adults *Am J Med* 57 747 1975
- 17 Osgood E E, Baker R L, Brownlee I E, Osgood M W, Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates of children four to seven years of age *Am J Dis Child* 58 61 1959
- 18 Osgood E E, Baker R L, Brownlee I E, Osgood M W, Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates for healthy children. Standards for children eight to fourteen years of age *Am J Dis Child* 58 782 1959
- 19 Rasch J R & Mogabgab W J Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia *Antimicrob Agents Chemother* 1965 693
- 20 Riski H & Alanko K *Mycoplasma* and influenza pneumonia in a series of 214 adults *Scand J Resp Dis Suppl* 89 235 1974
- 21 Stevens D, Swift P G F, Johnston P G B, Kearney P J, Corner B D & Burman D *Mycoplasma pneumoniae* infections in children *Arch Dis Child* 53 38 1978

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Fever, chills, cough, headache, general malaise and lethargy are prominent symptoms in both adults and older children with *M pneumoniae* infection (16) where is gastro intestinal and musculo skeletal symptoms though common are usually minor. Gastro intestinal symptoms occurred in 42% of all cases and were often severe enough to dominate the clinical picture. In patients under the age of five, cough, wheeze and vomiting were the most frequent specific symptoms.

At all ages, clinical signs are of limited diagnostic value. A previous study of children in a day care centre (7) suggested that lower respiratory tract involvement is rare in pre school children. The high proportion of pre school children with lower respiratory infection and radiological abnormalities in our series may be partly explained by the fact that few children in this age group are admitted to hospital solely on the basis of upper respiratory tract infection. Similar findings have been reported in a comparable hospital based series (21).

In a review of central nervous system manifestations of *M pneumoniae* infections, Hodges et al (12) found that in adults, septic meningitis, meningoencephalitis, psychosis, peripheral neuropathy, cerebellar ataxia, transverse myelitis and hemiplegia may be associated with respiratory disease. Cranial nerve mononeuropathy has also been reported (16). Similar findings have been described in children (14) where it was noted that patients under the age of 14 tend to be more severely affected than older patients though residual abnormalities were usually mild. Neurological symptoms or signs were present in 24 (23%) of our patients and occurred throughout childhood. Cerebrospinal fluid findings were consistent with previous reports (12-14). No attempt was made to detect antibodies or culture *M pneumoniae* in cerebrospinal fluid.

Mucocutaneous, musculo skeletal and haematological complications of *M pneumoniae* infection, although not uncommon in children (8), were seldom seen in the present series.

Like clinical signs, chest X rays and haematological investigations were of limited help in confirming the diagnosis. Foy et al (9) suggested that punctate mottling on chest X rays implying segmental or patchy consolidation and central dense infiltrates were helpful diagnostic clues, albeit not pathognomonic. In both adults (20) and children (21) the leucocyte count is normal or increased, with the neutrophils showing a shift to the left. We observed a similar trend but noted a depressed absolute lymphocyte count in 30% of our patients. An increase in the erythrocyte sedimentation rate in many of our patients accords with previous experience (20-21).

Mixed infection or bacterial superinfection associated with *M pneumoniae* is said to be uncommon (6-11). Nineteen (19%) of our patients also had viral infections but no associations with a specific viral agent was encountered.

Lack of response to antibiotic therapy, usually with ampicillin or penicillin, was one of the main indications for hospital referral. Even so, most patients were treated with these antibiotics in hospital. This suggests that hospital staff did not believe that antibiotic therapy had been adequate, or that they did not consider the possibility of *M pneumoniae* infection. Erythromycin and tetracycline have been held to be effective in treatment (19) yet only six patients were treated primarily with these antibiotics, either by their family doctor or in hospital.

Illnesses caused by *M pneumoniae* vary from asymptomatic to severe though there have been no reports of fatalities in children. Clinical recovery appears to be complete. In our short term follow up of patients with pulmonary involvement, chest X rays had invariably returned to normal within three months. It has been shown previously that functional recovery may be delayed (13). Our long term follow up findings (15) confirm that functional abnormalities may be present some years following apparent recovery, suggesting the possibility of residual lung damage.

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REFERENCES

- Altman P. L. & Dittmer D. S. *Blood and other body fluids*. Federation of American Societies for Experimental Biology, Washington D.C. 1961.
- Baernstein H. D., Trevisan E., Axtell S. & Quiligan J. J. *Mycoplasma pneumoniae* (Eaton atypical pneumonia agent) in children's respiratory infections. *J. Pediatrics* 66: 879, 1965.
- Bain H. H., Stewart S. & Simpson H. Infections due to *Mycoplasma pneumoniae* in children. *Arch. Dis. Child* 46: 739, 1971.
- Bradstreet H. P. & Taylor C. E. D. Technique of complement fixation test applicable to the diagnosis of viral disease. *Monthly Bulletin of Ministry of Health and Public Health Laboratory Service* 71: 96, 1966.
- Chanock R. M., Fox H. H., James W. D., Custer R. H., White R. J. & Senterfit L. B. Epidemiology of *Mycoplasma pneumoniae* infection in military recruits. *Ann. N.Y. Acad. Sci.* 143: 484, 1967.
- Denny F. W., Clyde W. A. & Glezen W. P. *Mycoplasma pneumoniae* disease: Clinical spectrum, pathophysiology, epidemiology and control. *J. Infect. Dis.* 173: 74, 1971.
- Fernald G. W., Collier A. M. & Clyde W. A. Jr. Respiratory infections due to *Mycoplasma pneumoniae* in infants and children. *Pediatrics* 55: 377, 1975.
- Foy H. M. & Alexander E. R. *Mycoplasma pneumoniae* infections in childhood. In: I. Schulman (ed.), *Advances in Pediatrics* 16: 301. Book Medical Publishers, 1969.
- Foy H. M., Loop J., Clarke E. R., Mansy A. W., Spence W. F., Feigl P. & Grayston J. T. Radiographic study of *Mycoplasma pneumoniae* pneumonia. *Am. Rev. Resp. Dis.* 108: 469, 1973.
- Glezen W. P., Thornburg G., Chin T. Y. D. & Wenner H. A. Significance of *Mycoplasma* infections in children with respiratory disease. *Pediatrics* 39: 516, 1967.
- Hers J. F. P. & Masurel N. Infections with *Mycoplasma pneumoniae* in civilians in the Netherlands. *Ann. N.Y. Acad. Sci.* 143: 447, 1967.
- Hodges G. R., Fass H. G. & Saslaw S. Central nervous system disease associated with *Mycoplasma pneumoniae* infection. *Arch. Intern. Med.* 130: 277, 1970.
- Kjellman B. Pulmonary function in children with *Mycoplasma pneumoniae* pneumonia. *Infection* 4: Suppl. 1: 71, 1976.
- Lerer R. J. & Kalavsky M. Central nervous system disease associated with *Mycoplasma pneumoniae* infection. Report of five cases and review of literature. *Pediatrics* 57: 658, 1973.
- Iok J. Y. Q., Waugh P. M. & Simpson H. *Mycoplasma pneumoniae* infection—A follow up study of 50 children with respiratory illnesses. Accepted for publication by *Arch. Dis. Child*, November 1978.
- Murray H. W., Masur H., Senterfit L. H. & Roberts H. B. The protean manifestations of *Mycoplasma pneumoniae* infection in adults. *Am. J. Med.* 52: 742, 1975.
- Osgood E. E., Baker R. L., Brownlee I. E., Osgood M. W., Ellis D. M. & Cohen W. Total differential and absolute leukocyte counts and sedimentation rates of children four to seven years of age. *Am. J. Dis. Child* 58: 61, 1939.
- Osgood E. E., Baker R. L., Brownlee I. E., Osgood M. W., Ellis D. M. & Cohen W. Total differential and absolute leukocyte counts and sedimentation rates for healthy children. Standards for children eight to fourteen years of age. *Am. J. Dis. Child* 58: 782, 1939.
- Rasch J. R. & Mogabgab W. J. Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia. *Antimicrob. Agents Chemother.* 1965: 693, 1966.
- Riski H. & Alanko K. *Mycoplasma* and influenza pneumonia in a series of 214 adults. *Scand. J. Resp. Dis. Suppl.* 89: 735, 1974.
- Stevens D., Swift P. G. F., Johnston H. G. B., Kearney P. J., Corner H. D. & Burman D. *Mycoplasma pneumoniae* infections in children. *Arch. Dis. Child* 53: 38, 1978.

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Fever, chills, cough, headache, general malaise and lethargy are prominent symptoms in both adults and older children with *M. pneumoniae* infection (16) where is gastro intestinal and musculo skeletal symptoms though common are usually minor. Gastro intestinal symptoms occurred in 42% of all cases and were often severe enough to dominate the clinical picture. In patients under the age of five, cough, wheeze and vomiting were the most frequent specific symptoms.

At all ages, clinical signs are of limited diagnostic value. A previous study of children in a day care centre (7) suggested that lower respiratory tract involvement is rare in pre school children. The high proportion of pre school children with lower respiratory infection and radiological abnormalities in our series may be partly explained by the fact that few children in this age group are admitted to hospital solely on the basis of upper respiratory tract infection. Similar findings have been reported in a comparable hospital based series (21).

In a review of central nervous system manifestations of *M. pneumoniae* infections, Hodges et al. (12) found that in adults aseptic meningitis, meningo encephalitis, psychosis, peripheral neuropathy, cerebellar ataxia, transverse myelitis and hemiplegia may be associated with respiratory disease. Cranial nerve mononeuropathy has also been reported (16). Similar findings have been described in children (14) where it was noted that patients under the age of 14 tend to be more severely affected than older patients though residual abnormalities were usually mild. Neurological symptoms or signs were present in 24 (23%) of our patients and occurred throughout childhood. Cerebrospinal fluid findings were consistent with previous reports (12, 14). No attempt was made to detect antibodies or culture *M. pneumoniae* in cerebrospinal fluid.

Mucocutaneous, musculo skeletal and haematological complications of *M. pneumoniae* infection although not uncommon in children (8) were seldom seen in the present series.

Like clinical signs, chest X rays and haematological investigations were of limited help in confirming the diagnosis. Foy et al. (9) suggested that punctate mottling on chest X rays implying segmental or patchy consolidation and central dense infiltrates were helpful diagnostic clues, albeit not pathognomonic. In both adults (20) and children (21) the leucocyte count is normal or increased with the neutrophils showing a shift to the left. We observed similar trend but noted a depressed absolutely lymphocyte count in 30% of our patients. An increase in the erythrocyte sedimentation rate in many of our patients accords with previous experience (20, 21).

Mixed infection or bacterial superinfection associated with *M. pneumoniae* is said to be uncommon (6, 11). Nineteen (19%) of our patients also had viral infections but no associations with a specific viral agent was encountered.

Lack of response to antibiotic therapy usually with ampicillin or penicillin was one of the main indications for hospital referral. Even so, most patients were treated with these antibiotics in hospital. This suggests that hospital staff did not believe that antibiotic therapy had been adequate or that they did not consider the possibility of *M. pneumoniae* infection. Erythromycin and tetracycline have been held to be effective in treatment (19) yet only six patients were treated primarily with these antibiotics either by their family doctor or in hospital.

Illnesses caused by *M. pneumoniae* vary from asymptomatic to severe though there have been no reports of fatalities in children. Clinical recovery appears to be complete. In our short term follow up of patients with pulmonary involvement, chest X rays had invariably returned to normal within three months. It has been shown previously that functional recovery may be delayed (13). Our long term follow up findings (15) confirm that functional abnormalities may be present some years following apparent recovery suggesting the possibility of residual lung damage.

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REFERENCES

- 1 Altman P L & Dittmer D S *Blood and other body fluids* Federation of American Societies for Experimental Biology Washington D C 1961
- 2 Baernstein H D Trevisani E Axtell S & Qualigan J J *Mycoplasma pneumoniae* (Eaton atypical pneumonia agent) in children's respiratory infections *J Pediatr* 66 873 1965
- 3 Bain H H Stewart S & Simpson H Infections due to *Mycoplasma pneumoniae* in children *Arch Dis Child* 46 739 1971
- 4 Bradstreet S M P & Taylor C E D Technique of complement fixation test applicable to the diagnosis of viral disease *Monthly Bulletin of Ministry of Health and Public Health Laboratory Service* 21 96 1967
- 5 Chanock R M Fox H H James W D Cutler R R White R J & Senterfit L B Epidemiology of *Mycoplasma pneumoniae* infection in military recruits *Ann NY Acad Sci* 143 484 1967
- 6 Denny F W Clyde W A & Glezen W P *Mycoplasma pneumoniae* disease Clinical spectrum pathophysiology epidemiology and control *J Infect Dis* 123 74 1971
- 7 Fernald G W Collier A M & Clyde W A Jr Respiratory infections due to *Mycoplasma pneumoniae* in infants and children *Paediatrics* 55 37 1975
- 8 Foy H M & Alexander E R *Mycoplasma pneumoniae* infections in childhood In I Schulman (ed) *Advances in Pediatrics* 16 301 Book Medical Publishers 1969
- 9 Foy H M Loop J Clarke E R Mansy A W Spence W F Feigl P & Grayston J T Radiographic study of *Mycoplasma pneumoniae* pneumonia *Am Rev Resp Dis* 108 469 1973
- 10 Glezen W P Thornburg D Chin T Y D & Henner H A Significance of *Mycoplasma* infections in children with respiratory disease *Pediatrics* 39 316 1967
- 11 Hers J T P & Masurel W Infections with *Mycoplasma pneumoniae* in children in the Netherlands *Ann NY Acad Sci* 143 447 1967
- 12 Hodges G R Foss R G & Saslaw M Central nervous system disease associated with *Mycoplasma pneumoniae* infection *Arch Intern Med* 130 277 1972
- 13 Kjellman B Pulmonary function in children with *Mycoplasma pneumoniae* pneumonia *Infection* 4 Suppl 1 71 1976
- 14 Lerer E J & Kalavsky S M Central nervous system disease associated with *Mycoplasma pneumoniae* infection Report of five cases and review of literature *Pediatrics* 57 658 1973
- 15 Lok I Y Q Waugh P R & Simpson H *Mycoplasma pneumoniae* infection—A follow up study of 50 children with respiratory illnesses Accepted for publication by *Arch Dis Child* November 1978
- 16 Murray H W Masur H Senterfit L B & Roberts R B The protean manifestations of *Mycoplasma pneumoniae* infection in adults *Am J Med* 57 247 1975
- 17 Osgood E E Baker R L Brownlee I F Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates of children four to seven years of age *Am J Dis Child* 58 61 1959
- 18 Osgood E E Baker R L Brownlee I E Osgood M W Ellis D M & Cohen W Total differential and absolute leukocyte counts and sedimentation rates for healthy children Standards for children eight to fourteen years of age *Am J Dis Child* 58 282 1959
- 19 Rasch J R & Mogabgab W J Therapeutic effect of erythromycin on *Mycoplasma pneumoniae* pneumonia *Antimicrob Agents Chemother* 1965 693 1966
- 20 Riski H & Alanko K *Mycoplasma* and influenza pneumonia in a series of 214 adults *Scand J Resp Dis Suppl* 89 235 1974
- 21 Stevens D Swift P G F Johnston P G M Kearney P J Corner B D & Burman B *Mycoplasma pneumoniae* infections in children *Arch Dis Child* 53 38 1978

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T AND B LYMPHOCYTE SUBPOPULATIONS AND LEUKOCYTE TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE IN ENERGY PROTEIN UNDERNUTRITION

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ABSTRACT Chandra R. K. (Department of Pediatrics, Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland, Canada) T and B lymphocyte subpopulations and leukocyte terminal deoxynucleotidyltransferase in energy protein undernutrition. *Acta Paediatr Scand* 68 841 1979.—Children with energy protein undernutrition showed a reduction in the number of circulating T lymphocytes identified on the basis of their ability to form rosettes with sheep red blood cells. T cells with a receptor for IgM (T_H) were decreased whereas T cells with a receptor for IgG (T_H) were increased. Surface immunoglobulin bearing B cells were comparable in well nourished and malnourished subjects but the proportion of B₂ was increased in the latter. Null cells without the conventional markers of T or B cells were proportionately increased. Leukocyte terminal deoxynucleotidyl transferase activity was elevated in the majority of undernourished children and correlated with the proportion of null cells. The significance of these observations is discussed and it is suggested that null cells represent immature undifferentiated T lymphocytes.

KEY WORDS Malnutrition, T lymphocytes, B lymphocytes, terminal deoxynucleotidyl transferase, null cells, cellular immunity.

Nutritional deficiency enhances susceptibility to infectious disease (9-24). Besides the common causal factors of poverty, poor sanitation and personal hygiene, overcrowding and contaminated food and water, undernutrition impairs several facets of the immune response (10-11), thereby increasing vulnerability to disease produced by pathogenic microorganisms. Depression of cell mediated immunity is a common observation as evidenced by reduced cutaneous delayed hypersensitivity to ubiquitous recall antigens and sensitizing chemicals, decreased mitogen induced lymphocyte proliferation response and low numbers of rosette forming T lymphocytes in the peripheral blood (3-6, 12-14, 20-21, 23). Recent immunologic work has demonstrated the heterogeneity of human T lymphocytes with regard to cell surface receptors, histochemical characteristics, RNA content, response to phyto mitogens, sensitivity to corticosteroids and irradiation, distribution in organs, chemotaxis,

lymphokine production and immunoregulatory role (25). Alterations in the proportion of lymphocyte subsets have been reported in primary immunodeficiency (28) and in malignancy (25). There is no published report on changes in lymphocyte subpopulations of T and B cells in malnutrition in man or experimental animals.

MATERIAL AND METHODS

Subjects

Twenty-one young children aged 11-38 months were diagnosed to be suffering from energy protein undernutrition on the basis of clinical features (growth failure, loss of subcutaneous tissue, skin and hair changes), decreased weight for height and low serum transferrin less than 16 mg% (7-11). All the patients came from rural India and none had any obvious systemic disease.

Fifteen healthy subjects aged 17-36 months from the same community served as controls.

Mononuclear cells

Heparinized peripheral venous blood was collected and mononuclear cells were isolated by Ficoll Hypaque den-

Table 1 Lymphocyte subpopulations and levels of terminal deoxynucleotidyl transferase (TdT)

Values are given as mean \pm S.E.M. Significance of differences (p) was assessed statistically using Student's t test. NS = not significant

Group	No	Total leukocyte count (per mm ³)	Rosette forming T cells (%)	B cells (%)			Null cells (%)	TdT (units/10 ⁶ cells)
				B	B ₇	B _u		
Undernourished	21	6.85 \pm 1.471	47.3 \pm 3.9	9.1 \pm 1.1	4.2 \pm 0.8	5.7 \pm 0.9	30.7 \pm 2.6	11.34 \pm 2.4 [*]
Healthy	15	7.95 \pm 1.894	73.7 \pm 2.8	4.0 \pm 0.7	6.3 \pm 0.7	8.7 \pm 1.2	7.0 \pm 0.7	1.08 \pm 0.0 [*]
p		NS	<0.01	<0.05	NS	NS	<0.001	<0.001

sity gradient centrifugation. Cells were washed twice in Hanks balanced salt solution and resuspended in RPMI 1640 containing 15% heat inactivated fetal calf serum. Phagocytic cells were depleted by addition of lymphocyte separation reagent (Technicon) incubation at 37°C for 10 min and centrifugation at 400 \times for 20 min. Interface cells were washed twice with Hanks balanced salt solution and resuspended in RPMI 1640 at a concentration of 4 \times 10⁶ cells per ml.

T lymphocytes

Lymphoid cells were mixed with equal volume of 1% neuraminidase treated sheep red blood cells (SRBC) in 25% fetal calf serum. The mixture was incubated at 37°C for 5 min, centrifuged at 200 \times for 5 min and incubated again at 4°C for 1 hour. The cell pellets were resuspended by gentle agitation, kept at 4°C for 10 min and examined for the proportion of cells forming rosettes with SRBC.

Aliquots of rosetting T lymphocytes were separated from non T cells by density gradient centrifugation. SRBC were lysed with distilled water and MEM. T lymphocyte subpopulations were separated by employing ox red blood cells (ORBC) coated with anti ORBC antibodies of IgM or IgG class. Isolated T lymphocytes were mixed with an equal volume of 1% ORBC anti ORBC IgM for enumeration of T_H cells or ORBC anti ORBC IgG for enumeration of T_H cells. centrifuged at 200 \times for 5 min and incubated at 4°C for 1 hour. The cell pellets were resuspended by gentle agitation, kept at 4°C for 10 min and examined for the proportion of cells forming rosettes with ORBC.

B lymphocytes

Cells bearing surface membrane immunoglobulin (smIg) were recognized by speckled or crescent shaped fluorescence on staining with fluorescein conjugated goat anti serum raised against human IgG. IgA and IgM prepared in our laboratory (6-28). Mononuclear cells were incubated in culture medium at 37°C for one hour and then incubated with the conjugated antisera. After thorough washing the cell preparations were examined under a fluorescence microscope equipped with epillumination. Monocytes were identified and excluded by their ability to phagocytose latex particles. Furthermore duplicate sets of cell preparations were treated with trypsin and incubated in culture medium for 8 hours and restained to demonstrate newly produced immunoglobulin.

Terminal deoxynucleotidyl transferase (TdT)

Mononuclear cells were ruptured by freezing and thawing three times. The suspension was centrifuged at 8000 \times for 1 hour and the supernatant assayed for TdT activity by the method of Coleman et al. (13). The results were expressed in units of 10⁶ cells; one unit of activity being the polymerisation by TdT of 1 nmol of nucleotide in 1 hour.

RESULTS

The number of T lymphocytes was lower and the proportion of smIgA bearing B cells and null cells was increased in undernourished children compared with findings in well nourished matched controls (Table 1). These differences were statistically significant. The total number of leukocytes and the proportion of B cells were comparable in the two groups. The B_u cells were increased in the malnourished.

T lymphocytes with receptors for IgM were decreased ($p < 0.01$) and those with receptors for IgG were increased ($0.05 < p < 0.01$) in malnourished subjects (Fig. 1).

Leukocyte TdT activity was significantly higher in undernourished children ($p < 0.001$) and correlated with the proportion of null cells in the peripheral blood ($r = 0.78$, $p < 0.01$) (Fig. 2, Table 1).

DISCUSSION

We observed a marked increase in leukocyte TdT (terminal deoxynucleotidyl transferase DNA nucleotidyl transferase nucleoside triphosphate, DNA deoxynucleotidyl trans

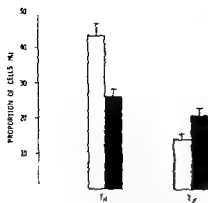


Fig. 1 T lymphocyte subpopulations with cell surface receptors for IgM (T_H) and IgG (T_F). Values are expressed as proportion of total T cell number and given as mean \pm S.D. Blocked columns represent undernourished children and open columns matched healthy controls.

ferase EC 2.7.7.31) in energy protein under nutrition. This enzyme is restricted in distribution to the thymus, bonemarrow and peripheral blood of a number of animal species (2, 27) which has been interpreted to indicate that it is involved in the functional differentiation of T cells and generation of immunological diversity and memory (2). It has been postulated that TdT may act as a somatic mutagen in lymphocytes (3). The intracellular location of the enzyme varies with the phase of the cell cycle with a prominent shift from the nucleus to cytoplasm during mitosis. In the thymus TdT positive cells are restricted to the cortex. There is a heterogeneity in the amount of the enzyme within different cells as demonstrated by immunofluorescence or biochemical assays (15). The majority of high and low density thymic cells contain little or no TdT whereas almost all medium density cortical thymocytes are TdT positive. It is possible that some cells contain antigenically inactive but functionally inactive enzyme. The critical role of the thymus in the differentiation of TdT positive lymphocytes is demonstrated by low enzyme activity in congenitally athymic nude (*nul/nul*) mice and in neonatally thymectomized mice. Thymosin has been shown to induce TdT activity in bone marrow cells of

nude mice (22). The contribution of thymus derived cells to the pool of TdT positive bone marrow cells is perhaps negligible (19). It is fascinating to note that both TdT positive and thymic antigen positive cells arise almost simultaneously during ontogenetic development although there are differences in the rate of evolution of these markers.

This study has demonstrated that nutritional deficiency is associated with a marked alteration in the number of lymphocyte subpopulations. The total number of smig bearing B lymphocytes was comparable in well nourished and malnourished groups although it was somewhat higher than the figures obtained in healthy well nourished subjects examined in Europe and North America. These differences may reflect the more frequent exposure of our study subjects to various microorganisms as a result of poor sanitation and heavily contaminated environment including water and food. The proportion of B cells bearing α heavy chain of immunoglobulin (B lymphocytes) was increased in the malnourished group.

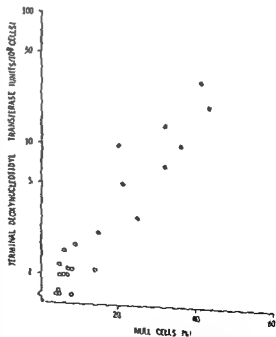


Fig. 2 Leukoocyte TdT activity related to the number of nucleated cells in undernourished (●) and healthy (O) groups.

Table 1 *Lymphocyte subpopulations and levels of terminal deoxynucleotidyl transferase (TdT)*

Values are given as mean \pm SEM. Significance of differences (*p*) was assessed statistically using Student's *t* test. NS = not significant.

Group	No	Total leukocyte count (per mm ³)	Rosette forming T cells (%)	B cells (%)			Null cells (%)	TdT (units/10 ⁶ cells)
				B _a	B _γ	B _μ		
Undernourished	21	6 852 \pm 1 471	47.3 \pm 3.9	9.1 \pm 1.1	4.2 \pm 0.8	5.7 \pm 0.9	30.7 \pm 2.6	11.34 \pm 2.4 [†]
Healthy	15	7 950 \pm 1 894	73.7 \pm 2.8	4.0 \pm 0.7	6.3 \pm 0.7	8.7 \pm 1.2	7.0 \pm 0.7	1.08 \pm 0.0
<i>p</i>		NS	<0.01	<0.05	NS	NS	<0.001	<0.001

sity gradient centrifugation. Cells were washed twice in Hanks balanced salt solution and resuspended in RPMI 1640 containing 15% heat inactivated fetal calf serum. Phagocytic cells were depleted by addition of lymphocyte separation reagent (Technicon). Incubation at 37°C for 10 min and centrifugation at 400 *g* for 20 min. Interface cells were washed twice with Hanks balanced salt solution and resuspended in RPMI 1640 at a concentration of 4 \times 10⁶ cells per ml.

T lymphocytes

Lymphoid cells were mixed with equal volume of 1% neuraminidase treated sheep red blood cells (SRBC) in 25% fetal calf serum. The mixture was incubated at 37°C for 5 min, centrifuged at 200 *g* for 5 min and incubated again at 4°C for 1 hour. The cell pellets were resuspended by gentle agitation, kept at 4°C for 10 min and examined for the proportion of cells forming rosettes with SRBC.

Aliquots of rosetting T lymphocytes were separated from non T cells by density gradient centrifugation. SRBC were lysed with distilled water and MEM. T lymphocyte subpopulations were separated by employing ox red blood cells (ORBC) coated with anti ORBC antibodies of IgM or IgG class. Isolated T lymphocytes were mixed with an equal volume of 1% ORBC anti ORBC IgM for enumeration of T_H cells or ORBC anti ORBC IgG for enumeration of T_γ cells. Centrifuged at 200 *g* for 5 min and incubated at 4°C for 1 hour. The cell pellets were resuspended by gentle agitation, kept at 4°C for 10 min and examined for the proportion of cells forming rosettes with ORBC.

B lymphocytes

Cells bearing surface membrane immunoglobulin (smIg) were recognized by speckled or crescent shaped fluorescence on staining with fluorescein conjugated goat anti serum raised against human IgG. IgA and IgM prepared in our laboratory (6, 28). Mononuclear cells were incubated in culture medium at 37°C for one hour and then incubated with the conjugated antisera. After thorough washing the cell preparations were examined under a fluorescence microscope equipped with epillumination. Monocytes were identified and excluded by their ability to phagocytose latex particles. Furthermore duplicate sets of cell preparations were treated with trypsin incubated in culture medium for 8 hours and restained to demonstrate newly produced immunoglobulin.

Terminal deoxynucleotidyl transferase (TdT)

Mononuclear cells were ruptured by freezing and thawing three times. The suspension was centrifuged at 80 000 *g* for 1 hour and the supernatant assayed for TdT activity by the method of Coleman et al. (13). The results were expressed in units of 10 cells; one unit of activity being the polymerization by TdT of 1 nmol of nucleotide in 1 hour.

RESULTS

The number of T lymphocytes was lower and the proportion of smIgA bearing B cells and null cells was increased in undernourished children compared with findings in well nourished matched controls (Table 1). These differences were statistically significant. The total number of leukocytes and the proportion of B cells were comparable in the two groups. The B_a cells were increased in the malnourished.

T lymphocytes with receptors for IgM were decreased (*p* < 0.1) and those with receptors for IgG were increased (0.05 < *p* < 0.1) in malnourished subjects (Fig. 1).

Leukocyte TdT activity was significantly higher in undernourished children (*p* < 0.001) and correlated with the proportion of null cells in the peripheral blood (*r* = 0.76, *p* < 0.01) (Fig. 2, Table 1).

DISCUSSION

We observed a marked increase in leukocyte TdT (terminal deoxynucleotidyl transferase, DNA nucleotidylexotransferase, nucleoside triphosphate: DNA: deoxynucleotidyl trans

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- 14 Ferguson A S Lawlor G J Jr Neumann C G Oh W & Stehm E R Decreased rosette forming lymphocytes in malnutrition and intrauterine growth retardation *J Pediatr* 85 717 1974
- 15 Goldschneider I Gregorie K E Barton R W & Bollum F J Demonstration of terminal deoxy nucleotidyl transferase in thymocytes by immunofluorescence *Proc Natl Acad Sci USA* 74 734 1977
- 16 Gross R L Reid J V O Newberne P M Burgess B Marston R & Hift W Depressed cell mediated immunity in megaloblastic anemia due to folic acid deficiency *Am J Clin Nutr* 28 775 1975
- 17 Gupta M Fernandes M Nair M & Good M A Spontaneous and antibody dependent cell mediated cytotoxicity by human T cell subpopulations *Proc Natl Acad Sci USA* 75 5137 1978
- 18 Harland P S E G Tuberculin reactions in malnourished children *Lancet* i 719 1965
- 19 Hutton J J & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in marrow of athymic nude mice *Blood* 49 1003 1976
- 20 MacDougall L G Anderson R McNab G M & Katz J Immune response in iron-deficient children impaired cellular defense mechanisms with altered humoral components *J Pediatr* 86 833 1975
- 21 Neumann C G Lawlor C G Stehm E R Swedheid M E Newton C Herbert J Ammann A J & Jacob M Immunological responses in malnourished children *Am J Clin Nutr* 28 89 1975
- 22 Pazmino N H Ihle J N & Goldstein A L Introduction *in vivo* and *in vitro* of terminal deoxy nucleotidyl transferase by thymosin in bone marrow cells from athymic mice *J Exp Med* 147 708 1978
- 23 Reddy V Bhaskaram C & Raghuramulu N Immunological responses in malnourished children *Indian Pediatr* 14 755 1977
- 24 Scrimshaw N S Taylor C E & Gordon J E *Interactions of nutrition and infection* World Health Organization Geneva 1968
- 25 Serrou B & Rosenfeld C *Human lymphocyte differentiation its application to human cancer* North Holland Amsterdam 1978
- 26 Smythe P M Breton Stiles G G Grace H J Mafoyane A Schonland M Coovadia H M Loening W E K Parent M A & Vos O H Thymolymphatic atrophy and depression of cell mediated immunity in protein-calorie malnutrition *Lancet* ii 939 1971
- 27 Sugimoto M & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in chick embryo lymphoid tissues *J Immunol* 122 39 1979
- 28 WHO Scientific Group Immunodeficiency Tech Rep Ser 630 World Health Organization Geneva 1978

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which may be the result of repeated gastro intestinal and respiratory infections in under nourished children

There was a significant decrease in the number of T lymphocytes with cell surface receptors for IgM (T_H) in malnutrition (Fig 1). These cells exert helper function in the differentiation of B lymphocytes to plasma cells. On the other hand the proportion of T cells with receptors for IgG (T_H) was increased. This subset of lymphocytes has recently been shown to mediate spontaneous lymphocyte mediated cytotoxicity and antibody dependent cell mediated cytotoxicity (17). Null cells also exert spontaneous lymphocyte mediated cytotoxicity. Both these activities had been demonstrated by us to be increased in malnourished children (6) in observation comparable with our present data of increase in T_H and null cell populations (Fig 1 Table 1). The biologic significance of these changes in T cell subsets are not yet clear. Nevertheless such alterations have been noted in primary immunodeficiency (28) and in cancer (25).

There was a significant reduction in the number of rosette forming T lymphocytes. This may be the result of a number of factors. Reduced cellular regeneration is the hallmark of protein deficiency (7) and may well be reflected in impaired proliferation of lymphoid cells. The frequent increase in free cortisol not bound to albumin in malnutrition may actually produce a lympholytic effect. T cells are susceptible to the influence of corticosteroids.

An alternative explanation for the reduction of rosetting T lymphocytes may lie in impaired maturation of T cell precursors. The various sequential steps of differentiation from progenitor T cell to mature T lymphocyte have been delineated recently (28). The acquisition of cell surface markers including the ability to rosette with SRBC is a relatively late event in differentiation. The marked increase in the proportion of null cells and a corresponding increase in leukocyte TdT activity suggests that many of the null cells are immature T lymphocytes. TdT is found almost exclusively in im-

mature T lymphocytes and is progressively lost as the cell differentiates to full maturity. TdT is also increased in acute lymphoblastic leukemia in which the blast cell has many of the morphologic and histochemical and surface membrane characteristics of immature lymphocytes. This is particularly true of null blast leukemia (R A Chandra unpublished data). The hypothesis that the reduction in rosetting T lymphocytes increase in null cells and elevated TdT activity in nutritional deficiency all point to impaired maturation of T lymphocytes is further supported by decreased thymic hormone levels in the serum of malnourished children (10a).

REFERENCES

- 1 Baltimore D. Is terminal deoxynucleotidyl transferase a somatic mutagen in lymphocytes? *Nature* 249: 409 1974.
- 2 Bollum J J. Terminal deoxynucleotidyl transferase. In P D Boyer (ed.) *The enzymes*. Academic Press New York 1974 vol 10 p 145.
- 3 Chandra R A. Immunocompetence in undernutrition. *J Pediatr* 91: 1184 1972.
- 4 Chandra R A. Rosette forming T lymphocytes and cell mediated immunity in malnutrition. *Br Med J* III: 604 1974.
- 5 Chandra R A. Fetal malnutrition and postnatal immunocompetence. *Am J Dis Child* 129: 450 1975.
- 6 Chandra R A. Lymphocyte subpopulations in malnutrition. Cytotoxic and suppressor cells. *Pediatrics* 59: 423 1977.
- 7 Chandra R A. Energy protein undernutrition. In V C Kelky (ed.) *Practice of pediatrics*. Harper & Row Hagerstown 1977 ch 32 p 1.
- 8 Chandra R A & Matsumura T. Ontogenetic development of the immune system and effects of fetal growth retardation. *J Immunol Med* 7: 1979. In press.
- 9 Chandra R A. Nutritional deficiency and susceptibility to infection. *Bull WHO* 57: 167 1979.
- 10 Chandra R A. Interactions of nutrition infection and immune response. Immunocompetence in nutritional deficiency: methodological considerations and intervention strategies. *Acta Paediatr Scand* 68: 137 1979.
- 10a Chandra R A. Serum thymic hormone activity in protein energy malnutrition. *Clin Exp Immunol* 1979. In press.
- 11 Chandra R A & Newberne P M. *Nutrition immunity and infection. Mechanisms of interactions*. Plenum New York 1977.
- 12 Chandra R A, Au H, Woodford G & Hyam P. Iron status, immunocompetence and susceptibility to infection. In *Ciba Foundation Symposium on Iron Metabolism*. Elsevier Amsterdam 1977 p 249.

- 13 Coleman M ■ Greenwood M F Hutton J J Bollum F J Lampkin B & Holland P Serial observations on terminal deoxynucleotidyl transferase activity and lymphoblast surface markers in acute lymphoblastic leukemia *Cancer Res* 36 1 ■ 1976
- 14 Ferguson A S Lawlor G J Jr Neumann C G Oh W & Stehlm E R Decreased rosette forming lymphocytes in malnutrition and intrauterine growth retardation *J Pediatr* 81 717 1974
- 15 Goldschneider I Gregorie K E Barton R W & Bollum F J Demonstration of terminal deoxy nucleotidyl transferase in thymocytes by immunofluorescence *Proc Natl Acad Sci USA* 74 734 1977
- 16 Gross R L Reid J V O Newberne P M Burgess B Marston R & Hift W Depressed cell mediated immunity in megaloblastic anemia due to folic acid deficiency *Am J Clin Nutr* 28 775 1975
- 17 Gupta S Fernandes G Nair M & Good ■ A Spontaneous and antibody dependent cell mediated cytotoxicity by human T cell subpopulations *Proc Natl Acad Sci USA* 75 5137 1978
- 18 Harland P S E G Tuberculin reactions in malnourished children *Lancet* i 719 1965
- 19 Hutton J J & Bollum F J Terminal deoxynucleotidyl transferase (TdT) in marrow of athymic nude mice *Blood* 48 1003 1976
- 20 MacDougall L G Anderson R McNab G M & Katz J Immune response in iron-deficient children: impaired cellular defense mechanisms with altered humoral components *J Pediatr* 86 833 1975
- 21 Neumann C G Lawlor C G Stehlm E ■ Swedseid M E Newton C Herbert J Ammann A J & Jacob M Immunological responses in malnourished children *Am J Clin Nutr* 28 89 1975
- 22 Pazmino N H Ihle J N & Goldstein A L Introduction *in vivo* and *in vitro* of terminal deoxy nucleotidyl transferase by thymosin in bone marrow cells from athymic mice *J Exp Med* 147 708 1978
- 23 Reddy V Bhaskaram C & Raghuramulu N Immunological responses in malnourished children *Indian Pediatr* 14 755 1977
- 24 Scrimshaw N S Taylor C E & Gordon J E *Interactions of nutrition and infection* World Health Organization Geneva 1968
- 25 Serrou B & Rosenfeld C *Human lymphocyte differentiation Its application to human cancer* North Holland Amsterdam 1978
- 26 Smythe P M Breton Stiles G G Grace H J Mafoyane A Schonland M Coovadia H M Loening W E K Parent M A & Vos G H Thymolymphatic atrophy and depression of cell mediated immunity in protein-calorie malnutrition *Lancet* ii 939 1971
- 27 Sugimoto M & Bollum F J Terminal deoxy nucleotidyl transferase (TdT) in chick embryo lymphoid tissues *J Immunol* 122 392 1979
- 28 WHO Scientific Group *Immunodeficiency* Tech Rep Ser 630 World Health Organization Geneva 1978

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THE SPECTRUM OF COW'S MILK ALLERGY IN CHILDHOOD

Clinical Gastroenterological and Immunological Studies

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ABSTRACT Hill D J, Davidson G P, Cameron H J H and Barnes G L (Department of Gastroenterology, Royal Children's Hospital, Melbourne, Australia). The spectrum of cow's milk allergy in childhood. *Clinical gastroenterological and immunological studies*. *Acta Paediatr Scand* 68: 847-1979. —Seventeen of 52 children suspected of having cow's milk allergy had this diagnosis confirmed after milk challenge in hospital. A broad spectrum of reactions was observed including skin eruptions, respiratory symptoms and gastrointestinal disturbance. Not all patients with gastrointestinal symptoms showed small bowel mucosal damage. Only patients with skin reactions had positive skin tests. IgA deficiency and IgE elevation were common. Four patients had symptoms within 3 days of birth. Twelve children tolerated cow's milk by three years of age. Cow's milk allergy can cause a variety of symptoms. Challenge with milk for several days may be required before allergic manifestations can be demonstrated.

KEY WORDS Cow's milk allergy, gastrointestinal tract, skin.

Allergy to cow's milk has been attributed to an immunological hypersensitivity to several proteins (22). There is evidence that specific humoral (19, 21) and cellular (3, 4) immune mechanisms may operate in cow's milk allergy (CMA), although their importance in the pathogenesis of milk hypersensitivity in individual patients is poorly understood. Several well defined syndromes have been associated with CMA (7, 10, 16, 31) but the multiplicity of poorly defined illnesses (7) the reported variability of the pathological lesion following milk ingestion in sensitized children (6, 16, 24) and the lack of a diagnostic laboratory test have led to either a reluctance to recognize CMA (9) or confusion with other more common causes of mild intolerance (5).

This study was designed to establish the spectrum of clinical disorders affecting the skin, respiratory and gastrointestinal tracts and to measure some immunological phenomena associated with CMA. As in other studies the diagnosis of CMA has rested on a reproducible response to cow's milk challenge, symptomatic improvement on withdrawal of

whole cow's milk protein from the diet and exclusion of other known causes of milk intolerance.

PATIENTS AND METHODS

The children whose investigations are reported here come from a group of 52 children who had gastrointestinal cutaneous or respiratory symptoms attributed to CMA by their parents and/or referring physician.

Initial outpatient investigations identified an alternative diagnosis in eight cases (sugar intolerance, infection, infestation, coeliac disease or milk aspiration). The remaining 44 children were admitted to hospital for investigation. Twenty-seven of them showed no response to milk challenge and were excluded from further consideration. The remaining 17 children reacted to cow's milk challenge in hospital and form the basis of this report. Eleven patients were breast fed for 3 to 14 months. Five were given only artificial feeds (patients 4, 6, 8, 11 and 16) and one (patient 5) had mixed feeds.

Each child was stabilised on a diet free from whole cow's milk proteins for at least four weeks before admission. The protocol followed is outlined in Fig 1 and has been previously described (5). The symptomatic response to milk provocation was defined as follows: immediate reactions occurred within one hour; intermediate reactions between one and 24 hours; and late reactions greater than 24 hours after commencing milk challenge. Patients with late reactions were ingesting normal volumes of cow's milk for their age at the time symptoms developed.

only a post challenge biopsy. The method used was that of Townley and Barnes (6). Duodenal juice and mucosal impression smears were examined for trophozoites of *Giardia lamblia* and tissue was prepared for histological examination and disaccharidase estimation (7). Structural changes were graded as showing normal appearance, mild, moderate or severe change as described by Townley et al. (7).

Other investigations. Patients with respiratory symptoms associated with cow's milk ingestion had a barium swallow to detect aspiration (30).

RESULTS

Symptomatic response to formal milk provocation. Eight of the 17 patients developed symptoms primarily involving the gastrointestinal tract but in only 3 was the reaction immediate (Table 1). Seven patients developed mainly skin reactions and this response was immediate in all. Three of the seven also developed diarrhoea and/or vomiting. Most cutaneous eruptions resolved within hours but

in one child (case 10) the rash persisted for 72 hours. Respiratory symptoms were predominant in two of the 17 children: cough, chest rattle and wheeze followed milk challenge. Two other patients (cases 9 and 10) gave a history of acute respiratory distress and skin eruptions following ingestion of milk at home but failed to reproduce these symptoms when challenged. Urticaria was elicited in these 2 patients after a small volume of milk was given and the challenge procedure ceased.

Four of the 17 children had a past history of pallor and shock consistent with an anaphylactic reaction. Two (patients 4 and 7) developed mainly gastrointestinal symptoms at the time of challenge; the other two had an immediate skin reaction as well as vomiting and/or diarrhoea.

Immune function studies. The results of

S-tase (≥ 11 units/g wet weight)		Skin test to milk	RAST to milk	IgE (IU/ml)	Other immune abnormalities	Other food allergies	Age of milk tolerance (months)
Before	After						
3	4.7	Neg	Neg	-		Nil	-
5	4.0	Neg	Neg	943	IgA < 5th ~	Nil	4
	4.4	Neg	Neg	40		Nil	0
3	1.3	Neg	Neg	0		Nil	16
	0	Neg	Neg	-		Nil	16
1	1.1	Neg	Neg	-		Soy	36
1	0.3	Neg	Neg	15		Nil	Intolerant
6	1.6	Neg	Neg	<10	IgA < 5th ~	Soy	Intolerant
4	6	Pos	Pos	-		Egg	Intolerant
9		Pos	Pos	158	IgA < 5th ~	Egg	13
17	5.8	Pos	Neg	46	IgA none detected	Nil	10
19	9	Pos	Neg	14	IgA < 5th ~	Nil	15
	-	Pos	Neg	381	IgA none detected	Egg	Intolerant
15	0	Pos	Neg	-	IgG > 95th ~	Lamb	18
1	4	Pos	Pos	-	IgG < 5th ~	Egg, Lamb, Peanut	Intolerant
10	0.5	Neg	Neg	100		Nil	36
	-			-		Nil	30

Skin sensitizing antibodies were found only in patients with cutaneous eruptions but not all of these had circulating IgE milk antibodies detected. Possibly short latency IgG antibodies (21) or incomplete saturation of peripheral IgE receptors accounts for these findings. Skin sensitizing antibodies often associated with anaphylactic reactions to allergens were detected in two of the children who had an anaphylactic reaction to cow's milk. However two others had no cutaneous reaction at the time of anaphylaxis nor at the time of formal milk challenge. Neither skin nor circulating IgE milk antibodies were present in the latter 2 cases. There is evidence that IgE mucosal hypersensitivity to allergens can occur without systemic or cutaneous involvement (11) but the possibility that other immune mechanisms may be operating in anaphylaxis cannot be excluded.

In a previous report (5) we attributed respiratory symptoms after milk ingestion to aspiration (30). Four patients in this study had a history of repeated respiratory symptoms after ingestion of cow's milk but not after soy formulae or other fluids. In 2 of the 4 wheezing occurred for several hours after challenge in hospital and this was reproduced on 3 occasions.

A marked discrepancy was noted between the severe symptoms reported by parents and those elicited by formal milk challenge. In hospital only the smallest volume of milk which elicited a response was given and the challenge ceased. In all cases this challenge volume of milk was much less than that given by parents at home. A variation in the volume of milk given in milk challenge by different investigators may account for the conflicting reports regarding the nature, rate of onset, frequency and severity of symptoms in CMA as well as differences in mucosal damage (6, 16, 24).

It has been suggested that breast feeding may prevent the development of some allergic diseases (18). Eleven of our patients were breast fed and had reactions after the first

known milk exposure. Two children (patients 8 and 11) had severe illnesses consistent with CMA within hours of birth and CMA was confirmed by challenge at 3 months of age. Possibly hypersensitivity to cow's milk may develop in utero (14) or cow's milk protein may be transmitted in breast milk (12). By the third year of life most children were able to ingest volumes of milk which previously caused symptoms. Some showed a dislike for milk but did not appear to continue to suffer from CMA. However CMA may persist beyond this age (8).

This study has clearly shown that there is no single laboratory test on which to base the diagnosis of CMA, and that the diagnosis still rests on a reproducible response to challenge under controlled conditions. Small bowel biopsy has an important role in excluding other non immune causes of gastrointestinal milk intolerance. It also provides a baseline for interpreting post challenge mucosal changes in patients with gastrointestinal symptoms in whom the response to milk is uncertain. Where a clear cut cutaneous respiratory or gastrointestinal response occurs after milk challenge a repeat small bowel biopsy appears to add little to the management of this disorder.

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REFERENCES

1. Ammann A. J. & Hong R. Selective IgA deficiency. Presentation of 30 cases and a review of the literature. *Medicine* 50: 233 (1971).
2. Barnes III L. & Townley R. R. W. Duodenal mucosal damage in 31 infants with gastroenteritis. *Arch Dis Child* 48: 343 (1973).
3. Borroni C., Dagna-Bricarelli F., Massimo L., Fossati-Guglielmoni A. & Durand P. Lymphocyte transformation in milk allergy. *Acta Paediatr Scand* 59: 449 (1970).

skin and RAST tests to cow's milk are shown in Table 1. No patient with predominantly gastrointestinal symptoms had evidence of skin sensitivity or circulating IgE antibodies to cow's milk. All seven patients who developed a skin eruption after milk ingestion had skin sensitizing antibodies present as evidenced by positive skin tests and 4 also had circulating IgE milk antibodies. One patient (case 16) with respiratory symptoms had skin sensitivity but not circulating IgE milk antibodies demonstrated. The other patient (case 17) had neither skin sensitivity nor circulating milk antibodies.

Six children had serum IgA levels below the 5th percentile for age and 1 patient had a serum IgG level below the 5th percentile (23). Total IgE was at least 100 IU/ml in 5 of the 10 patients in whom it was measured.

Haemoglobin levels and eosinophil counts were within the normal range in all patients.

Studies of gastrointestinal structure and function. Of the 8 children with predominantly gastrointestinal symptoms, 4 showed mucosal damage after milk challenge (Table 1). The mucosal abnormality was mild in 1 (case 5), moderate in 2 (cases 4 and 8) and severe in 1 (case 7). One of these patients (case 4) had mild mucosal abnormalities prior to challenge which became more marked after challenge. Only 1 of the 6 children with mainly cutaneous manifestations of CMA (case 11) showed duodenal mucosal changes after milk challenge. Two others (cases 9 and 11) had mild mucosal changes present before and after challenge.

One child (case 16) with predominantly respiratory symptoms had associated gastrointestinal symptoms. His duodenal mucosa showed mild non-specific abnormalities before and after milk challenge.

Lactase depression to half the prechallenge level occurred in 4 children with gastrointestinal symptoms (patients 4, 6, 7 and 8). The only child with respiratory symptoms after milk challenge to have a biopsy (case 16) had lactase depression noted before and after challenge. His gastrointestinal and re-

spiratory symptoms settled when fed with Glucose Nutrigen (Mead Johnson).

Age of onset and duration of CMA. Four children had symptoms consistent with CMA in the first 72 hours of life (cases 4, 8, 11 and 16) at the time of first milk ingestion in the neonatal nursery. Six other children had symptoms of CMA at the time of first known milk ingestion (cases 2, 7, 10, 13, 14 and 15). Their ages at this time ranged from 18 days to 14 months.

Twelve of the 17 patients became tolerant to cow's milk between 10 and 36 months of age (Table 1). Five patients were still unable to tolerate cow's milk at 7 to 28 months of age.

Associated food allergy. Soy, egg or lamb hypersensitivity was seen in association with CMA in 7 patients (Table 1).

DISCUSSION

Gastrointestinal, respiratory and cutaneous reactions have each been described in cow milk protein hypersensitivity (7, 10, 31). An association between IgA deficiency and high IgE levels (1, 15) and the limitations of skin testing to diagnose CMA (5) have been reported. This paper describes the spectrum of these associations in 17 children with proven CMA.

In contrast to other reports (25) small bowel mucosal damage was not seen in all patients with gastrointestinal symptoms. This may be because the lesion in CMA is patchy as in gastroenteritis (2) or detectable only by electron microscopy (24) but the degree of mucosal damage is also likely to be related to the amount of antigen administered during challenge.

Little attention has been given to the gastrointestinal symptoms which develop 2 hours or more after commencing cow's milk ingestion (16). Such late reactions occurred in 4 of our patients emphasizing the need to continue cow's milk challenge for several days in children with suspected CMA.

A PROSPECTIVE STUDY OF COW'S MILK PROTEIN INTOLERANCE IN SWEDISH INFANTS

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ABSTRACT Jakobsson I and Lindberg T (Department of Paediatrics Malmö General Hospital Malmö Sweden) A prospective study of cow's milk protein intolerance in Swedish infants *Acta Paediatr Scand* 68 853 1979—1079 of 1548 newborn infants were followed during their first year 328 were prospectively contacted once a month 751 were followed up at child welfare clinics Altogether 20 were diagnosed as being cow's milk protein intolerant (1.9%) Symptoms from the gastrointestinal tract and the skin predominated Only 2 had respiratory symptoms Ten had their symptoms within one week after the introduction of cow's milk 3 of them at their first cow's milk-containing meal A further 4 already had symptoms when fed only human milk The others (6 infants) showed symptoms after more than one week on a cow's milk containing diet Before 2 years of age 13 had recovered Twelve of the cow's milk protein intolerant infants also showed adverse reactions to other foods soy protein intolerance being the most common (7 infants) A family history of allergy was found in 35% (116) of the 328 infants and in 70% (14) of those with cow's milk protein intolerance

KEY WORDS Cow's milk cow's milk protein intolerance food intolerance

Adverse effects from foods are on record from ancient times Hippocrates observed that cow's milk could cause gastric upset and urticaria (4) but food hypersensitivity was rarely reported before von Pirquet introduced the concept of allergy in 1906 (32) Since 1966 the term allergy has been used mostly to describe IgE mediated hypersensitivity reactions (18, 21) It is still not known how many hypersensitivity reactions to cow's milk proteins are mediated Therefore we prefer the term cow's milk protein intolerance meaning any adverse reaction whether of immediate or delayed type to cow's milk proteins giving symptoms in the gastrointestinal tract the skin or the respiratory tract

Estimates of the incidence of cow's milk protein intolerance in infants have produced considerably varying figures from 0.3% to 7.5% (1, 5, 6, 13, 15, 31) This variation may be due to differences in the composition of the clinical materials to varying diagnostic criteria used for the diagnosis and also to a variation in the incidence from one area to another

This study determined the incidence of cow's milk protein intolerance in infancy in a Swedish urban community its various manifestations and the course of the disease during the first years of life

PATIENTS AND METHODS

Malmö a town in southern Sweden has about 250 000 inhabitants About 2 500 infants are born each year It has one obstetric and one children's hospital During the period of this study 45% of the infants in Malmö were entirely breastfed at 3 months and 0% at 6 months (L. Righard M.D. Head of Child Welfare Clinics in Malmö personal communication) From 9 February to 24 March 1976 and 11 October 1976 to 17 April 1977 all women with a newborn infant at the obstetric hospital were informed by one of us (I.J.) about cow's milk protein intolerance They received both oral and written information about the design of the study and were invited to participate in it

During these periods 1555 infants were born alive 7 died neonatally Of the other 1548 the parents of 33 agreed to participate Four failed to fulfil the study (Fig. 1) This group of 38 comprise Group A Of the other 1510 751 (G or P B) were followed up via records at child welfare clinics attended by physicians from the children's hospital or practitioner paediatricians The physicians and the nurses at the child welfare clinics were informed

- 4 Brostoff J Cellular hypersensitivity in milk allergy *Clin Allergy* 1 350 1970
- 5 Davidson G P Hill D J & Townley R R W Gastrointestinal milk allergy in childhood A rational approach *Med J Aust* 1 945 1976
- 6 Fontain J L & Navarro J Small intestinal biopsy in cow's milk protein allergy in infancy *Arch Dis Child* 50 357 1975
- 7 Freier S Pediatric gastrointestinal allergy *Clin Allergy* 3 597 1973
- 8 Goldman A S Anderson D W Jr Sellers W A Superstein S Kniker W T Hulpert S R & Collaborators Milk allergy I Oral challenge with milk and isolated milk proteins in allergic children *Pediatrics* 52 425 1963
- 9 Harees J Paediatric gastrointestinal allergy In J Brostoff (ed) *Clinical immunology allergy in paediatric medicine* Blackwell Oxford 1973 p 128
- 10 Heimer D C Seirs J W & Kniker W T Multiple precipitins to cow's milk in chronic respiratory disease *Am J Dis Child* 103 634 1967
- 11 Huggins K G & Brostoff J Local production of specific IgE antibodies in allergic rhinitis patients with negative skin test *Lancet* II 148 1975
- 12 Jakobsson I & Lindberg T Cow's milk as a cause of infantile colic in breast fed infants *Lancet* I 437 1978
- 13 Johansson S G Bennich H & Berg T In vitro diagnosis of atopic allergy 3 Quantitative estimation of circulating IgE antibodies by the radioallergen sorbent test *Int Arch Allergy* 41 443 1971
- 14 Kaufman H S Allergy in the newborn skin test reactions confirmed by the Prausnitz-Kustner test at birth *Clin Allergy* 1 363 1971
- 15 Kjellmann N Johansson S G O & Roth A Serum IgE levels in healthy children quantitated by a sandwich technique *Clin Allergy* 6 51 1976
- 16 Kuitunen P Visakorpi J K Savilahti F & Pelkonen P Malabsorption syndrome with cow's milk intolerance Clinical findings and course in 54 cases *Arch Dis Child* 50 351 1975
- 17 Mancini G Carbonaro A O & Heremans J F Immuno-chemical quantitation of antigens by single radial immunodiffusion *Immunochemistry* 3 235 1966
- 18 Matthew D J Taylor B Norman A P Turner M W & Soothill J F Prevention of eczema *Lancet* I 321 1977
- 19 Matthews T E & Soothill J F Complement activation after milk feeding in children with cow's milk allergy *Lancet* II 893 1970
- 20 Mueller H L Weiss R J O'Leary D & Murray A B The incidence of milk sensitivity and the development of allergy in infants *N Engl J Med* 268 1220 1963
- 21 Parish W F Detection of reaginic and short term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons *Clin Allergy* 1 369 1971
- 22 Roy C & Dubois R Gastroenterologic disorders In R F Stiehm & V A Fulaniti (eds) *Immunologic disorders in infants and children* Saunders Philadelphia 1973 p 387
- 23 Shelton M J Meek F Goller I & Hosking C S Serum immunoglobulin levels in children *Aust J Med Technol* 5 113 1974
- 24 Shiner M Brilard J & Smith M E Small intestinal mucosa in cow's milk allergy *Lancet* I 176 1975
- 25 Sumithran E & Iyngkaran N Is jejunal biopsy really necessary in cow's milk protein intolerance? *Lancet* II 1172 1977
- 26 Townley R R W & Barnes G L Intestinal biopsy in childhood *Arch Dis Child* 48 450 1973
- 27 Townley R R W Khaw K T & Schwachman H Quantitative assay of disaccharidase activities of small intestinal mucosal biopsy specimens in infancy and childhood *Pediatrics* 36 911 1965
- 28 Walker W A & Isselbacher K J Intestinal antibodies *N Engl J Med* 297 767 1977
- 29 Wide L & Porath J Radioimmunoassay of proteins with the use of sephadex-coupled antibodies *Biochem Biophys Acta* 130 257 1966
- 30 Williams H E & Freeman M Milk inhalation pneumonia The significance of fat filled macrophages in tracheal secretions *Aust Paediatr J* 9 296 1973
- 31 Wilson J F Lahey M E & Heimer D C Studies on iron metabolism V further observations on cow's milk induced gastrointestinal bleeding in infants with iron-deficiency anaemia *J Pediatr* 84 335 1974

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ABSTRACT Jakobsson I and Lindberg T (Department of Paediatrics, Malmö General Hospital, Malmö, Sweden). A prospective study of cow's milk protein intolerance in Swedish infants. *Acta Paediatr Scand* 68: 853, 1979. — 1 079 of 1 548 newborn infants were followed during their first year. 328 were prospectively contacted once a month. 701 were followed up at child welfare clinics. Altogether 20 were diagnosed as being cow's milk protein intolerant (1.9%). Symptoms from the gastrointestinal tract and the skin predominated. Only 2 had respiratory symptoms. Ten had their symptoms within one week after the introduction of cow's milk. 3 of them at their first cow's milk-containing meal. A further 4 already had symptoms when fed only human milk. The others (6 infants) showed symptoms after more than one week on a cow's milk containing diet. Before 2 years of age 13 had recovered. Twelve of the cow's milk protein intolerant infants also showed adverse reactions to other foods, soy protein intolerance being the most common (7 infants). A family history of allergy was found in 35% (116) of the 328 infants and in 70% (14) of those with cow's milk protein intolerance.

KEY WORDS Cow's milk, cow's milk protein intolerance, food intolerance.

Adverse effects from foods are on record from ancient times. Hippocrates observed that cow's milk could cause gastric upset and urticaria (4) but food hypersensitivity was rarely reported before von Pirquet introduced the concept of allergy in 1906 (32). Since 1966 the term allergy has been used mostly to describe IgE mediated hypersensitivity reactions (18, 21). It is still not known how many hypersensitivity reactions to cow's milk proteins are mediated. Therefore we prefer the term cow's milk protein intolerance meaning any adverse reaction, whether of immediate or delayed type, to cow's milk proteins giving symptoms in the gastrointestinal tract, the skin, or the respiratory tract.

Estimates of the incidence of cow's milk protein intolerance in infants have produced considerably varying figures, from 0.3% to 7.9% (1, 5, 6, 13, 15, 31). This variation may be due to differences in the composition of the clinical materials, to varying diagnostic criteria used for the diagnosis, and also to a variation in the incidence from one area to another.

This study determined the incidence of cow's milk protein intolerance in infancy in a Swedish urban community, its various manifestations, and the course of the disease during the first years of life.

PATIENTS AND METHODS

Malmö, a town in southern Sweden, has about 250 000 inhabitants. About 7 500 infants are born each year. It has one obstetric and one children's hospital. During the period of this study, 45% of the infants in Malmö were entirely breastfed at 3 months and 70% at 6 months (L. Righard, M.D., Head of Child Welfare Clinics in Malmö, personal communication). From 9 February to 24 March 1976 and 11 October 1976 to 17 April 1977, all women with a newborn infant at the obstetric hospital were informed by one of us (I.J.) about cow's milk protein intolerance. They received both oral and written information about the design of the study and were invited to participate in it.

During these periods, 1 555 infants were born alive, 7 died neonatally. Of the other 1 548, the parents of 337 agreed to participate. Four failed to fulfil the study (Fig. 1). This group of 338 comprised 61% of the other 1 16 751 (C.O.P.B.) were followed up via records at child welfare clinics attended by physicians from the children's hospital or practitioner paediatricians. The physicians and the nurses at the child welfare clinics were informed

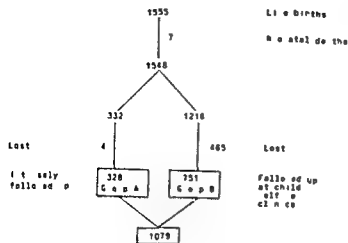


Fig. 1. Survey of the number of infants.

about the study. Altogether 1079 infants were followed up. Of the other 465 (see Fig. 1) one third had left the city, the records of one third could not be found, probably because of a change of surname, and of one third because of moving several times from one district to another within the city.

Follow-up examinations

Group A. Data were obtained from all parents concerning family history of allergy (eczema, urticaria, asthma, hay fever). Once a month the parents gave on a standardized form data concerning each infant's height and weight (obtained at the child welfare clinics) and type of feeding. Questions concerning problems were also answered (infantile colic, diarrhoea, vomiting, bronchitis, rhinitis, exanthema). The parents could telephone any day to discuss problems.

All infants were examined by one of us (I. J.) at age 3 and 12 months. Those with symptoms that might be ascribed to allergy were examined more frequently.

Group B. Infants suspected of cow's milk protein intolerance were admitted to the children's hospital and examined by us. We studied the records of the 751 infants at child welfare clinics with special regard to type of feeding, gain in weight and height, feeding difficulties, infantile colic, eczema, or recurrent bronchitis during their first year of life. Finally, the hospital records of those infants admitted because of any problem were also studied.

Managements of infants suspected of cow's milk protein intolerance

Cow's milk protein intolerance was suspected in any infant with one or more of the following symptoms: vomiting and/or diarrhoea not due to any other demonstrable cause; infantile colic not disappearing after advice on feeding technique or treatment with dimethylpolysiloxane (Minifom®); eczema, urticaria, and exanthemas without known relation to other foods; and recurrent bronchitis.

The infant was given a diet free from cow's milk—if possible human milk, otherwise a soy-based formula.

Those not tolerating soy protein were given a formula based on casein hydrolysate (Nutramigen®; Mead Johnson). Infants with symptoms when fed human milk initially continued at the breast with the mother on a cow's milk-free diet.

If the symptoms disappeared on a cow's milk protein-free diet, the infant was challenged with whole cow's milk. The challenges were performed after a period of cow's milk withdrawal varying from 4 to 70 weeks (mean 8.4 weeks). (In one infant it was performed after 7 weeks with a negative result.) Gradually increasing amounts of whole cow's milk were given every third hour, beginning with 1 ml and finishing with 50 ml. The challenge was completed when a distinct positive reaction was seen. At a suspected positive reaction, the last dose was repeated once. A symptom-free infant had a total of 176 ml cow's milk.

We made no challenges to diagnose food intolerance other than cow's milk protein intolerance. However, the parents spontaneously often performed challenges and the reactions were carefully recorded.

Diagnostic criteria

The diagnosis of cow's milk protein intolerance was based on (a) the presence of symptoms on a diet containing cow's milk, (b) the disappearance of symptoms on withdrawal of cow's milk, (c) at least two positive milk challenges with return of identical symptoms.

Lactose intolerance was excluded by a careful history by the infant tolerating human milk and in doubtful cases by a blood glucose rise of ≥ 1.1 mmol/l and no symptoms following a lactose tolerance test (dose 2 g/kg body weight).

RESULTS

Incidence

A total of 20 infants (11 boys and 9 girls) fulfilled the criteria given above and were diagnosed as cow's milk protein intolerant (Table 1). Five reacted within one hour after challenge and six after more than 24 h (24–96 h). Cow's milk proteins were eliminated from the diet in a further 30 because of suspected symptoms.

Table 1. Incidence of cow's milk protein intolerance

For explanation of groups A and B, see Fig. 1.

	No. of infants	%
Group A ($n=328$)	12	3.7
Group B ($n=751$)	8	1.1
Total ($n=1079$)	20	1.9

Table 2 *Symptomatology and course of the disease in 20 infants with cow's milk protein intolerance*

Patient	Introduction of cow's milk age (weeks)	Onset of symptoms age (weeks)	Elimination of cow's milk age (weeks)	Symptoms	Positive milk challenges age (weeks)	Tolerant to cow's milk age (weeks)	Intolerances to other foods
FA ♂	0	70	78	D B E	37 60 96	>104	Orange tomato soy protein
PS ♂	8	44	51	E	77 104	>104	Orange egg fish
VL ♀	16	3	3	C D V Ex E	8 74 77	>104	Wheat soy protein
FT ♂	Never	6	16	E	70 40 72	>104	Orange egg wheat nuts soy protein
AS ♀	10	74	74	C V U Ex E	78 57	>104	Fish soy protein
CA ♀*	16	16	70	V E	32 40 72 104	>104	Orange tomato
TV ♀	74	74	44	D	60 80	>104	-
MC ♂	18	18	74	V U Ex E	40 48	>104	Egg soy protein
AN ♂	0	4	4	Ex	8 1	20	-
DL ♂	17	76	78	U Ex	36 40	48	Several fruit juices
ET ♀	16	16	16	V Ex D	70 78	44	-
MH ♂	3	70	78	E	37 40	48	Orange rosehip egg fish
AK ♀	8	4	4	C V	8 16	28	-
TI ♀	5	6	17	C V	16 24	36	Rosehip soy protein
MD ♀*	8	8	8	V	16 40	60	-
NE ♂			9	V W	12 16	40	Soy protein
AR ♂	3	3	9	C V D W	16 20	78	-
PH ♀	7	4	4	C V	3 40	48	-
DS ♀	8	8	15	C U Ex	70 40	56	-
DE ♂	7		8	E B	12 24	5	Orange

V=vomiting D=diarrhoea C=infantile colic U=urticaria Ex=exanthema E=eczema B=bronchitis W=slow weight gain Italics=first symptom(s)

* Symptoms at first cow's milk containing meal

First symptoms when fed only human milk

toms. In 7 the elimination had no effect. In 23 the symptoms disappeared and did not return when the infants were challenged with cow's milk. They continued on a cow's milk based formula without any symptoms. They were followed for at least 2 months after the challenge, 12 up to one year of age.

Symptoms

Table 2 presents symptoms and course of the disease in the infants with cow's milk protein intolerance. Seven infants had symptoms from the gastrointestinal tract only, in 2 combined with a slow weight gain. One had diarrhoea as the predominant symptom, a 6-month old girl tolerating human milk, got diarrhoea and abdominal pain on cow's milk on several challenges. A lactose tolerance test was normal.

She had normal stools on a cow's milk free diet and no signs of intestinal infection. Secondary lactose intolerance could thus be excluded. Seven infants had infantile colic. In 3 of them colic was the only symptom at the debut, but was ignored and the infants were not admitted until other symptoms occurred. Five infants presented symptoms from the skin only, and 6 from both the skin and gastro-intestinal tract. Two had symptoms from the respiratory tract, but they also reacted from the skin and intestinal tract. Four of the infants had symptoms when fed only human milk. The symptoms disappeared with the mothers on a cow's milk free diet and returned with the mother on a normal diet. Identical symptoms appeared on direct challenge with whole cow's milk.

Table 3 Onset of symptoms in relation to the introduction of cow's milk in infants with cow's milk protein intolerance

	No. of infants
<1 week	10 ^a
1-4 weeks	2
>4 weeks	4

^a Four infants had their first symptoms when fed only human milk.

^b Three had their first symptoms at first cow's milk containing meal.

All 5 infants reacting within one hour after challenge vomited. 2 also had urticaria and exanthema, one colic and diarrhoea. Of the 6 infants reacting after more than 24 h after challenge 3 had eczema, 1 diarrhoea, 2 diarrhoea combined with colic and vomiting.

Onset of symptoms

Eleven infants had their first symptoms before 8 weeks, 8 between 12 and 26 weeks, and one boy at 44 weeks of age (Table 2). The latter had been fed cow's milk since 8 weeks of age but got cow's milk dependent eczema at 44 weeks of age. Infantile colic was the predominant symptom in the infants reacting before 8 weeks of age (6/11).

Table 3 shows that 10 infants got their symptoms within one week after the introduction of cow's milk. Three reacted on their first cow's milk containing meal.

Infants who became cow's milk protein intolerant were entirely breastfed as long as the other infants (13 weeks v. 12 weeks (mean values)).

Duration of the intolerance

Nine infants became cow's milk tolerant before 1 year of age, 3 of them before 11 months (Table 2). At 2 years of age a further 3 had recovered. Eight infants aged 2-3 years are still intolerant to cow's milk, 7 of them have multiple food intolerances and eczema.

Associated food intolerances

Twelve infants with cow's milk protein intolerance also showed intolerance to other foods (soy, egg, fish, wheat, tomato, orange, nuts). Seven were intolerant to soy protein which was the most common food intolerance associated with cow's milk protein intolerance. Ten infants had adverse reactions to several types of foods (see Table 2).

Intolerance to foods other than cow's milk

Adverse reactions to foods other than cow's milk (e.g. egg, fish, wheat, tomato, orange, strawberry) were reported in 26% (85) of the Group A infants. Many of these reactions were diffuse exanthemas after eating some kind of fruit. The reactions had occurred at least twice before we considered them as food dependent. These intolerance periods were usually brief— a few months.

Allergic heredity

A family history of allergy (mother, father and/or sibling) was found in 35% (116) of the 328 infants in Group A. In the 20 infants with milk protein intolerance the corresponding figure was 70% (14).

DISCUSSION

The diagnosis of cow's milk intolerance is still clinically based, so far there is no laboratory test which can distinguish one individual with this condition from others (3, 7, 8, 10, 12, 19, 26, 28).

The diagnosis must therefore be based on clinical observation of the effects of elimination and introduction of cow's milk. The original criteria for making the diagnosis of cow's milk protein intolerance according to Goldman (14) with three positive milk challenges is often impossible to fulfil. It is often unacceptable to make repeated milk challenges at short intervals. This procedure also probably leads to fewer diagnoses of the disorder, as the

infants can have become tolerant before the third challenge. Therefore most clinicians now would accept one or two positive challenges as strong evidence in favour of the diagnosis (33). We used two positive challenges as a diagnostic criterion.

It is important to exclude lactose intolerance as a cause of the infant's symptoms. Mostly this is easily done by a careful history. In only one infant in our study had a lactose tolerance test to be made. The results of lactose tolerance tests could be of value but must be interpreted with caution as up to 25% might be false positives (2, 17, 23). It is also evident that lactose intolerance might accompany cow's milk protein intolerance (16, 25). Moreover it has been reported that commercial lactose preparations might contain small amounts of cow's milk proteins (30).

Previous studies on the incidence of cow's milk protein intolerance gave figures varying from 0.3 to 7.5%. The incidence 0.3% is given by Collins Williams (6) after excluding all patients with major allergies and does not represent a normal population. A retrospective study by Bachman & Dees (1) found that 1% of 403 infants had cow's milk protein intolerance. Recently Stintzing & Zetterstrom retrospectively calculated an incidence of 0.8% (31). The incidence in two other prospectively studied groups of infants is reported to be 1.5% (15) and 7.5% (13). The overall incidence in our study was 1.9% but in the group most intensely studied (Group A) it was 3.7%. Naturally parents with allergy problems of their own are more interested in taking part in a study such as this. Therefore there is probably an inevitable selection in Group A with a 35% family history of allergy but this figure does not differ much from that reported by Kjellman (30%) in a Swedish unselected population (22). Moreover Group A probably includes infants with mild forms of cow's milk protein intolerance not otherwise diagnosed.

The symptomatology at the debut agrees with previous studies (1, 5, 11, 13). Gerrard et al. (13) found that about 40% of the infants

with cow's milk protein intolerance developed their symptoms within one week after their first exposure to cow's milk. In our study the corresponding figure is 14/20 (70%). Four had symptoms already when fed only human milk and three at their first cow's milk containing meal. This supports the hypothesis that cow's milk protein sensitization can occur *in utero* (27) or via human milk (9, 20, 29). A further 4 infants of Group A with severe infantile colic when fed only human milk were free of symptoms when their mothers had a cow's milk free diet (20). They were challenged several times with positive results by giving the mothers a normal diet. However when challenged directly with whole cow's milk at age 3-4 months they had no symptoms and thus did not fulfil our diagnostic criteria.

The frequency of soy protein intolerance in cow's milk protein intolerant infants in our study (35%) agrees with other reports (11) but is higher than that of Kuitinen et al. (24) who studied infants with malabsorption syndrome because of cow's milk intolerance.

26% of Group A had adverse reactions to foods other than cow's milk. Of the infants with cow's milk protein intolerance the corresponding figure was 60%. It is important to bear in mind this increased tendency towards associated food intolerances also reported by others (11, 13, 24) when introducing new foods in the diet for the cow's milk protein intolerant infant.

Gerrard et al. (13) found that 27% had lost their sensitivity to cow's milk before 2 years of age. Clein (5) reports as many as 95% before 2 years. Our corresponding figure is 60%. The future prospects of those who are cow's milk protein intolerant in infancy are obscure. No infant of our study has so far been followed for more than 3 years. According to Clein (5) 80% of infants intolerant to cow's milk develop other allergic manifestations before they reach the age of puberty.

In conclusion the incidence of cow's milk protein intolerance in infancy in a Swedish urban community was 20 out of c. 1000. In 14

of the 20 the onset of symptoms occurred within one week after first exposure to cow's milk. Thirteen of the 20 recovered from the intolerance before 2 years of age.

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REFERENCES

- Bichman A D & Dees S C. Milk allergy. I. Observations on incidence and symptoms in well babies. *Pediatrics* 20: 391 1959.
- Berg N O, Borulf S, Jakobsson I & Lindberg T. How to approach the child suspected of malabsorption. *Acta Paediatr Scand* 67: 403 1978.
- Berg N O, Jakobsson I & Lindberg T. Do pre and postchallenge small intestinal biopsies help to diagnose cow's milk protein intolerance? *Acta Paediatr Scand* 68: 657 1979.
- Chobot R. *Pediatric Allergy*. New York: McGraw Hill Book Co Inc 1951.
- Clark N W. Cow's milk allergy in infants and children. *Int Arch Allergy* 13: 245 1959.
- Collins Williams C. The incidence of milk allergy in pediatric practice. *J Pediatr* 49: 39 1956.
- Dannreus A, Johansson S G O, Foucard T & Ohlin S. Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 66: 31 1977.
- Dannreus A. Food allergy in childhood. A study of the humoral immune response to basic food antigens in relation to clinical symptoms. Thesis. *Acta Univ Upsal* 312 1979.
- Donnelly H H. The question of the elimination of foreign protein (egg white) in woman's milk. *J Immunol* 19: 15 1910.
- Editorial. Cow's milk protein and the small intestine. *Lancet* I: 702 1978.
- Freier S. Paediatric gastrointestinal allergy. *Clin Allergy* 3: 597 1973.
- Fallstrom S P, Ahlstedt S & Hanson L Å. Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl Immunol* 56: 97 1978.
- Gerrard J W, Mackenzie J W A, Goluboff N, Garson J Z & Maningas C S. Cow's milk allergy. Prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* Suppl 734 1973.
- Goldman A S, Anderson D W Jr, Sellers W A. Superstein S, Kniker W T & Hilpern S R. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 77: 425 1961.
- Hilpern S R, Sellers W A, Johnson R B, Anderson D W, Superstein S & Rensch J S. Development of childhood allergy in infants fed breast, soy or cow's milk. *J Allergy Clin Immunol* 51: 139 1973.
- Harrison M, Kirby A, Walker Smith J A, Franke N F & Wood C B. Cow's milk protein intolerance: a possible association with gastroenteritis, lactose intolerance and IgA deficiency. *Br Med J* 1: 1501 1976.
- Harrison B M & Walker Smith J A. Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity and lactose tolerance tests. *Gut* 18: 48 1977.
- Ishizuka K, Ishizuka T & Hornbrook M M. Physicochemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as a carrier of reaginic activity. *J Immunol* 97: 15 1966.
- Jyngkärin N, Robinson M J, Sumithran S K, Lam S K, Puzhuchery S D & Yadir M. Cow's milk protein sensitive enteropathy. An important factor in prolonging diarrhoea of acute infective enteritis in early infancy. *Arch Dis Child* 53: 150 1978.
- Jakobsson I & Lindberg T. Cow's milk as a cause of infantile colic in breastfed infants. *Lancet* II: 437 1978.
- Johansson S G O, Bennich H & Wide L. A new class of immunoglobulin in human serum. *Immunology* 14: 265 1969.
- Kjellman N I M. Immunoglobulin E and atopy in childhood. *Linköping University Medical Dissertations* No 36 1976.
- Krasilnikoff P A, Gudmund Hoyer E & Moltke H H. Diagnostic value of disaccharide tolerance tests in children. *Acta Paediatr Scand* 64: 693 1975.
- Kuitunen P, Visakorpi J K, Savilathi E & Pelkonen P. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 50: 351 1975.
- Lin H Y, Tsao M U, Moore B & Giday Z. Bovine milk protein-induced intestinal malabsorption of lactose and fats in infants. *Gastroenterology* 54: 27 1967.
- Matthews T S & Soothill J F. Complement activation after milk feeding in children with cow's milk allergy. *Lancet* II: 893 1970.
- Matsumura T, Kuroume T, Oguri M, Iwasaki I, Kanbe Y, Yamada T, Kawabe S & Negishi K. Egg sensitivity and eczematous manifestations in breastfed newborns with particular reference to maternal sensitization. *Ann Allergy* 35: 222 1975.
- Savilathi E. Immunochemical study of the malabsorption syndrome with cow's milk intolerance. *Gut* 14: 400 1973.

- 9 Shannon W R Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs *Am J Dis Child* 22 223 1971
- 10 Spies J R New antigens in lactose *Proc Soc Exp Biol Med* 137 711 1971
- 11 Stuntz G & Zetterstrom R Cow's milk allergy incidence and pathogenetic role of early exposure to cow's milk formula *Acta Paediatr Scand* 68 383 1979
- 12 Von Pirquet C Allergie *Munch Med Wochenschr* 51 1457 1906
- 13 Walker Smith J Cow's milk protein intolerance Transient food intolerance of infancy *Arch Dis Child* 50 347 1975

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REFERENCES

1. Bichman A D & Dees S C. Milk allergy: I. Observations on incidence and symptoms in well babies. *Pediatrics* 20: 393 1959.
2. Berg N O, Borulf S, Jakobsson I & Lindberg T. How to approach the child suspected of malabsorption. *Acta Paediatr Scand* 67: 403 1978.
3. Berg N O, Jakobsson I & Lindberg T. Do pre and postchallenge small intestinal biopsies help to diagnose cow's milk protein intolerance? *Acta Paediatr Scand* 68: 657 1979.
4. Chabot R. *Pediatric Allergy*. New York: McGraw Hill Book Co Inc 1951.
5. Clein N W. Cow's milk allergy in infants and children. *Int Arch Allergy* 13: 245 1958.
6. Collins Williams C. The incidence of milk allergy in pediatric practice. *J Pediatr* 48: 39 1956.
7. Dannaeus A, Johansson S G O, Foucard T & Ohman S. Clinical and immunological aspects of food allergy in childhood. I. Estimation of IgG, IgA and IgE antibodies to food antigens in children with food allergy and atopic dermatitis. *Acta Paediatr Scand* 66: 31 1977.
8. Dannaeus A. Food allergy in childhood. A study of the humoral immune response to basic food antigens in relation to clinical symptoms. Thesis. *Acta Univ Upsal* 312 1979.
9. Donnelly H H. The question of the elimination of foreign protein (egg white) in woman's milk. *J Immunol* 19: 15 1930.
10. Editorial. Cow's milk protein and the small intestine. *Lancet* I: 702 1978.
11. Freier S. Paediatric gastrointestinal allergy. *Clin Allergy* 3: 597 1973.
12. Fallstrom S P, Ahlstedt S & Hanson L A. Specific antibodies in infants with gastrointestinal intolerance to cow's milk protein. *Int Arch Allergy Appl Immunol* 56: 97 1978.
13. Gerrard J W, Mackenzie J W A, Goluboff N, Garson J Z & Maningas C S. Cow's milk allergy: Prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* Suppl 734 1973.
14. Goldman A B, Anderson D W Jr, Sellers W A, Superstein S, Kniker W T & Halpern S R. Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 37: 425 1963.
15. Halpern S R, Sellers W A, Johnson R B, Anderson D W, Superstein S & Reisch J S. Development of childhood allergy in infants fed breast soy or cow's milk. *J Allergy Clin Immunol* 51: 139 1973.
16. Harrison M, Kilby A, Walker Smith J A, Franke N L & Wood C B S. Cow's milk protein intolerance: a possible association with gastroenteritis, lactose intolerance and IgA deficiency. *Br Med J* I: 1501 1976.
17. Harrison B M & Walker Smith J A. Reinvestigation of lactose intolerant children: lack of correlation between continuing lactose intolerance and small intestinal morphology, disaccharidase activity and lactose tolerance tests. *Gut* 18: 48 1977.
18. Ishizuka K, Ishizuka T & Hornbrook M M. Physicochemical properties of human reaginic antibody. IV. Presence of a unique immunoglobulin as carrier of reaginic activity. *J Immunol* 97: 75 1966.
19. Iyengar N, Robinson M J, Sumthran S, Lam S K, Puzhucherry S D & Yadav M. Cow milk protein sensitive enteropathy: An important factor in prolonging diarrhoea of acute infectious enteritis in early infancy. *Arch Dis Child* 53: 140 1978.
20. Jakobsson I & Lindberg T. Cow's milk as a cause of infantile colic in breastfed infants. *Lancet* II: 431 1978.
21. Johansson S G O, Bennich H & Wide L. A new class of immunoglobulin in human serum. *Immunochemistry* 14: 265 1969.
22. Kjellman N I M. Immunoglobulin E and atopic allergy in childhood. *Linköping University Medical Dissertations* No 36 1976.
23. Krasilnikoff P A, Gudmund Hoyer E & Molik H H. Diagnostic value of disaccharide tolerance tests in children. *Acta Paediatr Scand* 64: 693 1975.
24. Kuitunen P, Visakorpi J K, Savilathi E & Pekonen P. Malabsorption syndrome with cow's milk intolerance. Clinical findings and course in 54 cases. *Arch Dis Child* 50: 751 1975.
25. Lin H Y, Tsao M U, Moore H & Giday Z. Bovine milk protein—induced intestinal malabsorption of lactose and fats in infants. *Gastroenterology* 54: 27 1967.
26. Matthews T S & Soothill J F. Complement activation after milk feeding in children with cow milk allergy. *Lancet* II: 893 1970.
27. Matsumura T, Kurume T, Oguri M, Iwasaki I, Kanbe Y, Yamada T, Kawabe S & Negishi K. Egg sensitivity and eczematous manifestations in breastfed newborns with particular reference to maternal sensitization. *Ann Allergy* 35: 277 1975.
28. Savilathi E. Immunochemical study of the malabsorption syndrome with cow's milk intolerance.

- 9 Shannon W R Demonstration of food proteins in human breast milk by anaphylactic experiments on guinea pigs *Am J Dis Child* 77 773 1971
- 10 Spies J R New antigens in lactose *Proc Soc Exp Biol Med* 137 711 1971
- 11 Stintzing G & Zetterstrom R Cow's milk allergy in infancy and pathogenetic role of early exposure to cow's milk formula *Acta Paediatr Scand* 68 383 1979
- 12 Von Pirquet C Allergie *Munch Med Wochenschr* 53 1457 1906
- 13 Walker Smith J Cow's milk protein intolerance Transient food intolerance of infancy *Arch Dis Child* 50 347 1975

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CONGENITAL PERSISTENT PROXIMAL TYPE RENAL TUBULAR ACIDOSIS IN TWO BROTHERS¹

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ABSTRACT Winsnes A Monn E Stokke O and Feyling T (Depts of Paediatrics and Clinical Chemistry Rikshospitalet Oslo and Dept of Paediatrics Fylkesjukhuset i Kristiansund Kristiansund Norway) Congenital persistent proximal type renal tubular acidosis in two brothers *Acta Paediatr Scand* 68 861 1979 —Two brothers showed severe and persistent hyperchloraemic metabolic acidosis (capillary blood pH 7.07–7.14) due to a low renal bicarbonate threshold at 11 mmol/l. The maximal tubular capacity for bicarbonate reabsorption was reduced to about half the normal. A high dose of acetazolamide (25 mg/kg) lowered the tubular bicarbonate reabsorption substantially indicating the presence of carbonic anhydrase. Both the glomerular filtration rate, the renal blood flow and the renal concentrating capacity were slightly reduced. The clinical characteristics were growth retardation, mental retardation, nystagmus, corneal opacities, cataract, glaucoma and enamel defects of the permanent teeth. Serum thyroxine was pathologically low without clinical signs of hypothyreosis. The erythrocytes showed an increased osmotic resistance. Autopsy of the younger brother who died at 4 1/2 years old revealed thyroid and thymus weights of 25% of the normal. The kidney tubular cells were swollen with vacuoles. The glomeruli had a normal appearance.

KEY WORDS Renal tubular acidosis, bicarbonate wasting, growth retardation, mental retardation, cataract, glaucoma, nystagmus.

At least two physiologically distinct tubular disorders may give rise to renal tubular acidosis (RTA) (18, 19). The classical or distal RTA is characterized by an inability of the distal tubule to generate a sufficiently large hydrogen ion gradient between blood and the tubular fluid (7, 8, 10, 17, 20). Thus the excretion of ammonium ions and titratable acid are reduced and urinary pH is usually above 6.5 despite overt acidosis. In the proximal or bicarbonate wasting type of RTA, on the other hand, excretion of acid in the distal tubules is normal and the urine is normally acidic with a pH down to 5 during acidosis. In this type of RTA, an impaired ability to reabsorb bicarbonate in the proximal tubules causes the hyperchloraemic acidosis (18, 19).

In the majority of cases of proximal RTA, multiple dysfunctions of the proximal tubules are present (Fanconi syndrome) and the renal

disorder is secondary to a systemic disease, e.g. galactosaemia, tyrosinaemia, cystinosis etc. (8, 10, 20). The so called primary form of proximal RTA, where no systemic disease can be demonstrated and where no tubular dysfunction except bicarbonate wasting is present, usually appears as a transitory defect in male infants with growth failure as the main clinical feature (11).

A primary familial form of persistent proximal RTA was recently described (1). The affected family members were however asymptomatic and the only clinical finding was short stature (1). The purpose of this article is to report the unusual clinical characteristics of another form of proximal RTA that is persistent, hereditary and apparently primary.

A short presentation of this condition was given at the Tenth Annual Meeting of the European Society for Paediatric Nephrology in Barcelona 1976.

METHODS AND PROCEDURES

Renal tubular function studies were performed on the patient B H at the age of 10 years. Bicarbonate reabsorption was studied by infusion of sodium bicarbonate (250 mmol/l) at rates gradually increasing from 25 to 75 mmol/hour. Inulin was infused from a separate flask for the determination of GFR.

Urine was collected from an urethral catheter at 30 min intervals and the pH was measured immediately at 37°. Blood samples were obtained from an indwelling venous cannula at midpoint during each period of urine collection.

Titrimble acid in urine is the amount of base equivalents necessary for adjustment to the actual pH of the blood. Clinical chemical data were obtained with conventional methods.

CASE REPORTS

The family

The family consists of the unrelated parents and three boys: B H, O H and S H. With the exception of B H and O H there is no history of mental or growth retardation and no occurrence of acidosis, nystagmus, cataract or glaucoma. The father was born in 1945. His height is 181 cm. The mother was born in 1944. Her height is 167 cm. She has two sisters and their parents have diabetes mellitus.

Clinical histories of the patients B H and O H

B H and O H were born after normal pregnancies at term in 1966 and 1968 respectively with birth weights of 4050 and 3670 g and length 51 cm. They could both walk at 18 months. The growth of B H and O H was retarded during the first year but later their heights have been at -2 SD. The speech development of both B H and O H has been retarded. At the age of 12 years B H can speak incomplete sentences with 4-5 words. He has learned some of the letters of the alphabet but cannot read.

The severe metabolic acidosis was diagnosed at 5 and 3½ years of age respectively and has been unchanged since.

Both patients have experienced several episodes of inappropriately severe symptoms with sopor and vomiting after slight to moderate head traumas. The degree of acidosis has been unchanged on these occasions. O H died 4½ years old during such an episode lasting three days after a moderate head trauma. At autopsy of the brain there was fresh bleeding in the corpus callosum and in the right temporal lobe.

Both patients had undulating nystagmus from the neonatal period and both had slight milky white opacities in the cornea when first examined by an ophthalmologist at 5 and 3½ years of age. Cataract in the rear part of the lens has been noted in B H since he was 9 years. A steady progress leading to severely reduced vision will necessitate enucleation in the near future. At eleven years bilateral glaucoma was diagnosed immediately operated and later treated with pilocarpine and timolol eye drops.

Laboratory data for B H and O H The range of data

from both patients, whereas mean values are given in parenthesis for B H and O H respectively. Capillary blood pH 7.07-7.15 (7.12-7.11), standard bicarbonate 7-11 (10.9) mmol/l, pCO₂ 2.9-4.3 (3.5-3.9) kPa. Serum concentrations in mmol/l: Na 135-140 (138-138), Cl 114-124 (122-120), K 3.2-4.1 (3.7-3.3), Ca 1.1-1.3 (1.1-1.1), P 1.5-1.7 (1.6-1.6), urea 5.7-8.4 (7.5-6.7) and creatinine 44-71 (53-71) µmol/l.

Haematological parameters in B H and O H respectively at the time when the osmotic fragility of erythrocytes was measured: Hgb 13.1 and 10.4 g/100 ml, Hct 42 and 35%, MCV 90 and 99 fL, MCH 28 and 29 fL, MCHC 31 and 30 g/100 ml, reticulocytes 5% and 7.4%, thrombocytes 123 and 172 × 10⁹/l.

Serum concentrations measured at least once with normal results in B H: Mg, Zn, Pb, uric acid, lactate, pyruvate, amino acids, bilirubin, haptoglobin, folic acid, iron, total iron binding capacity, vitamin B₁₂, alkaline phosphatase, transaminases, lactic dehydrogenase, creatine kinase, total cholesterol, triglycerides, phospholipids.

Hormones in B H: Parathormone, calcitonin and growth hormone were normal. Aldosterone in serum was either normal or moderately increased, whereas aldosterone excretion in the urine was normal. Plasma insulin values were 30 and 49 µU/ml despite normal blood glucose and lack of obesity.

Thyroid function Thyroxine values in B H were 37.39 and 45 nmol/l at 5.9 and 10 years, and in O H at 4 years thyroxine was 54 nmol/l despite treatment with L-thyroxine. Free thyroxine index and protein bound iodine were likewise pathologically low in both patients, whereas triiodothyronine, TSH and thyroxine binding globulin were normal.

Erythrocyte enzymes in B H: Analyses of 8 different erythrocyte enzymes associated with haemolytic anaemia revealed activities higher than normal, possibly due to a somewhat younger mean cell age. The total carbonic anhydrase activity was normal. The lipid composition of the erythrocyte membranes was also normal as was the scanning electron microscopic appearance.

Sweat electrolytes in B H were normal except for an increased chloride concentration at 80 mmol/l.

Gastric acid production increased normally upon stimulation with histamine.

Urine analyses The pH was around 5. Protein, amino acids, lysozyme, citrate and calcium were excreted in normal amounts. Cyclic AMP excretion increased normally upon stimulation with parathormone. Glucosuria occurred only after increasing the blood glucose level to above 14 mmol/l. Alpha ketoglutarate was usually excreted in large amounts, up to 300 mg/day in the urine of both patients. Otherwise the gas chromatograms of the organic acids in urine were normal.

Autopsy findings in O H: The thyroid gland was smaller than normal (1 g as against 4 g normal weight at this age). The microscopic examination of the thyroid gland showed a somewhat reduced follicle epithelium but no obvious pathological findings. The thymus weight was also low about 25% of normal but without significant microscopic changes. In the kidneys the glomeruli appeared normal. In the proximal tubular epithelium cells vacuolization and increased fat content was observed.

Table 1 Renal function parameters of B H

Test	Patient	Normal
Creatinine clearance (ml/min) (1.73 m ²)	47.68 70 ■	70-130
Inulin clearance (ml/min) (1.73 m ²) ^a	55 61 61	90-150
Effective renal plasma flow (¹²⁵ I Hippuran) (ml/min) (1.73 m ²)	364	450-650
Tubular reabsorption of phosphate	70 77 83	80-95
Titrateable acid (mmol/min) (1.73 m ²)	17 21 25 31	4-16
Ammonium excretion (μmol/min) (1.73 m ²)	8 9 1 22	6-26
Urine pH	4.9-5.1	
Osmolality mOsm/kg	800-1000	
Concentrating capacity mOsm/kg water deprivation + Vasopressin	740-780	(814-1374)

RESULTS AND DISCUSSION

Acid base and electrolyte metabolism

B H and O H have exhibited a marked hypochlorhaemic metabolic acidosis that in the case of B H has been unchanged during an observation period of 7 years. Urinary pH has constantly been around 5 and excretion of both titrateable acid and ammonium were in the normal range in B H (Table 1).

During bicarbonate infusion the urinary pH increased immediately when the serum level of bicarbonate started to increase (Fig. 1). No bicarbonate was found in the urine in the basal condition whereas large amounts were excreted at a normal serum bicarbonate level (Fig. 2) increasing the urinary pH to around 8 (Fig. 1).

Our patients thus clearly belong to the proximal or bicarbonate wasting category of RTA. The bicarbonate threshold of B H was found to be 11 mmol/l (Figs 1 & 2) which is considerably lower than that reported by Soriano and coworkers (18) in primary proximal RTA where thresholds in the range 18-20 mmol/l were found. However the 20-months old girl described by Donckerwolcke et al (2) had a bicarbonate threshold expressed as total CO₂ in serum of 10.3 mmol/l which was similar to that of B H.

The tubular capacity for reabsorption of bicarbonate was essentially unchanged at serum bicarbonate levels between 14 and 26 mmol/l (Fig. 2). The maximal tubular transport capa-

city for bicarbonate (Tm-HCO₃) was 1.29, 1.13 and 1.14 mmol per 100 ml of glomerular filtrate (GFR) in the bicarbonate infusion studies this being similar to the girl reported by the Dutch group (2) but again much lower than that reported in the American patients (18).

The Tm-HCO₃ values observed may be lower than the true capacity since expansion of the extracellular volume which is unavoidable during bicarbonate infusion will lower bicarbonate reabsorption (12). However this error will be of similar magnitude in the clinical studies reported (2, 18) the differences observed thus being significant. In addition the theoretical error in Tm-HCO₃ is probably of minor importance since the total

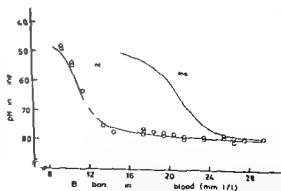


Fig. 1 Relationship of urinary pH to actual bicarbonate in venous blood in B H during NaHCO₃ infusion. The normal curve (drawn by inspection) represents the mean response of healthy infants as described by Soriano et al (18).

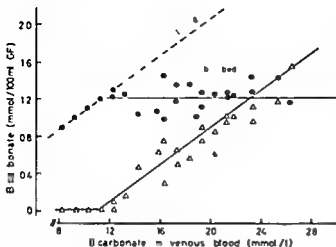


Fig. 2 Relation between venous blood concentration renal tubular reabsorption and urinary excretion of bicarbonate in B. H. The stippled line represents the theoretical line of 100% reabsorption. Filled symbols represent reabsorbed and open symbols excreted bicarbonate.

amount of fluid given during the bicarbonate infusion studies in B. H. was similar to the volume of urine voided (1500–1800 ml). Furthermore, the serum creatinine concentration remained remarkably constant 53–49–51 mmol/l during the bicarbonate infusion. Serum Na^+ increased from 136 to 145 mmol/l.

The bicarbonate infusion normalized both serum bicarbonate and chloride, whereas serum potassium decreased rapidly from 3.5 to a dangerously low level at 2 mmol/l. This precipitous serum K^+ fall is probably due to an increased Na^+ - K^+ exchange within the distal tubules presented with great amounts of NaHCO_3 . Bicarbonate administration is known to increase the serum level of parathyroid hormone leading to phosphaturia (5), possibly explaining the development of pronounced hypophosphatemia with a fall from 2.1 to 0.23 mmol/l.

In healthy individuals with normal bicarbonate levels, the proximal tubules reclaim 85–90% of the filtered bicarbonate. The enzyme carbonic anhydrase present both in the brush border and the interior of the proximal tubular cells is thought to be responsible for most of this reabsorption (7, 17). Thus, when the carbonic anhydrase inhibitor acetazolamide is given, an increased bicarbonate excretion in

urine normally occurs. Such a response represents indirect evidence for the presence of functional carbonic anhydrase. The patients of Sorrento et al. (18) doubled their bicarbonate excretion after a single dose of acetazolamide, in contrast to the patient from Holland (7) where no definite response was found even with a dose of 25 mg/kg. This latter finding was taken to indicate the absence of normal carbonic anhydrase. Our patient exhibited no increase in bicarbonate excretion at an ordinary dose of acetazolamide. On the contrary, a somewhat lowered excretion was found (Table 2). When, however, the acetazolamide dose was doubled (25 mg/kg), a significant decrease of Tm-HCO_3^- and thus an increase of bicarbonate excretion occurred at both serum bicarbonate levels around 10 (data not presented) and above 20 mmol/l (Table 2). Responsive carbonic anhydrase seems thus to be present in B. H. A quantitative or qualitative defect in its activity might nevertheless be present, especially as the response to an ordinary acetazolamide dose was abnormal. Enzymatic and electron microscopic studies

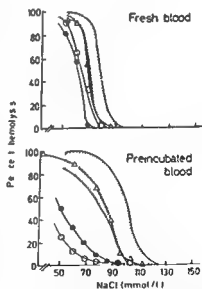


Fig. 3 Osmotic fragility of erythrocytes. Heparinized blood was incubated for 2 hours at room temperature with various dilutions of buffered saline. The test was performed within 30 min after blood collection and after 24 hours incubation of the blood at 37°C. Circles represent the patients B. H. and O. H. and triangles their normal brother S. H.

Table 2 The effect of acetazolamide on bicarbonate reabsorption at near normal serum bicarbonate level

Sodium bicarbonate was infused at a constant rate of 0.33 mmole per min during both studies I and II. Acetazolamide (Diamox[®]) was given intravenously at the start of collection period 4.

Study no	30-min period	Acetazolamide (mg/kg b.w.)	Serum (mmol/l)		Diuresis (ml/min)	GFR (ml/min)	Bicarbonate (mmol/100 ml GF reabsorbed/excreted)	
			HCO ₃	K				
I	1	-	2	2.4	2.5	26	1.72	0.98
		-	23	2.4	3.2	28	1.76	1.04
	3	-	24	2.4	3.3	26	1.27	1.13
	4	15	6	2.1	2.8	20	1.69	0.91
	5	1	27	2.1	2.7	2	2.23	0.47
II	1	-	21	2.4	4.2	28	1.15	0.95
		-	21	-	5.0	38	1.13	0.97
	3	-	22	2.3	4.7	33	1.13	1.07
	4	25	23	2.3	6.7	29	0.52	1.78
	5	25	25	-	7.9	40	0.97	1.53
	6	25	5	2.3	7.8	34	0.77	1.73

could clarify this question but we have not found it justified to take a kidney biopsy.

One of the unique ways in which the kidney can provide H⁺ for acid excretion or reabsorption of HCO₃⁻ is by decarboxylation of alpha ketoglutarate producing CO₂. One may speculate whether the increased excretion of alpha ketoglutarate found in our patients is due to lowered decarboxylation of this compound in this type of RTA where less than half the normal amount of HCO₃⁻ is presented to the tubules for reabsorption. In favour of this is the finding of reduced alpha ketoglutarate excretion during treatment of B.H. with alkali (see below).

Parathormone is known to reduce proximal bicarbonate reabsorption by a mechanism other than inhibition of carbonic anhydrase possibly indirectly by a reduction of the proximal sodium and fluid absorption (14). The important role of parathormone in regulation of HCO₃⁻ reabsorption is shown by the occurrence of systemic acidosis due to bicarbonate wastage in patients with hyperparathyroidism (9, 16). Our patient B.H. had normal serum levels of parathormone and no clinical signs of hyperparathyroidism. Administration of parathormone (6 IU/kg) resulted in a 3 fold increased diuresis, 2 fold in

creased GFR and 7 fold increased natriuresis but no bicarbonaturia which agrees well with an inhibited reabsorption of sodium being the primary event (14).

Both the endogenous creatinine and inulin clearances were moderately decreased as was the effective renal plasma flow as estimated by ¹³¹I Hippuran renography (Table 1). The urinary concentrating ability was also slightly reduced (Table 1). The reason for these reductions is not clear. Intravenous pyelography was normal. Microscopic examination of the kidneys of O.H. did not reveal glomerular or collecting duct pathology nor any sign of interstitial calcinosis as frequently found in distal but not usually in proximal RTA (10, 20). Contraction of the extracellular fluid volume which might have been beneficial because of its effect on bicarbonate reabsorption (12) is probably not causative as normalization of GFR was not observed during bicarbonate infusion (Table 2). Theoretically slight hypokalaemia could explain the reduced concentrating ability. However improvement was not observed during treatment with K₂ citrate. Habitually increased drinking was not causative as diuresis was normal (Table 1).

Attempts to correct the acidosis of B.H. by peroral medication with K₂-citrate (14 mmol x

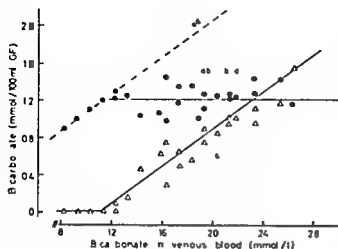


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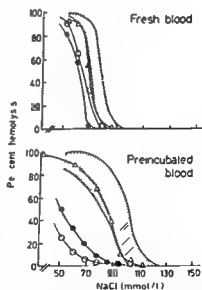


Fig. 3 Osmotic fragility of erythrocytes. Heparinized blood was incubated for 2 hours at room temperature with various dilutions of buffered saline. The test was performed within 30 min. after blood collection and after 74 hours incubation of the blood at 37°C. Circles represent the patients B. H. and O. H. and triangles their normal brother S. H.

21 days against 24 days as the lower normal limit. This is similar to the findings of Pavone et al. (13) in a patient with Lowe's syndrome. They suggested an extra erythrocytic factor as being responsible. However the finding of abnormally increased osmotic resistance of the erythrocytes (Fig. 3) would in our patients probably favour a change in some intrinsic property of the erythrocyte membrane itself. This is because incubation of normal erythrocytes in the patients' serum did not result in increased osmotic resistance, neither did incubation of the patients' erythrocytes with normal serum lower the osmotic resistance. In this connection it is of interest that Morris et al. (8) because of the association of familial RTA and elliptocytosis suggest the possibility that the cell membrane of renal tubular cells and erythrocytes share a common protein.

Increased osmotic resistance has been described both in iron deficiency anaemia, thalassaemia and cholestasis (3). In these conditions the erythrocytes have a greater than normal membrane surface area in relation to the intracellular volume allowing a greater than normal volume expansion before bursting. MCHC was just below the lower normal limit in our patients possibly explaining some of the increased osmotic resistance. There were no signs of cholestasis and electrophoresis of haemoglobin from H₁H was normal. We also found no increase of the erythrocytic cholesterol phospholipid ratio as in target cells (3).

CONCLUSION

It is clear that the degree of acidosis in primary proximal RTA may vary greatly from one patient to another. In our siblings both the acidosis and the clinical consequences are severe.

Except for growth retardation the clinical and biochemical characteristics of our patients are different from those of other published patients with either proximal or distal RTA although several features are similar to those of

Lowe's syndrome (12, 13, 20). In the oculo-cerebrorenal syndrome of Lowe however multiple tubular defects (Fanconi syndrome) are present and the acidosis is not as severe as in our patients.

The clinical condition in our patients is suggestive of a generalized enzyme or membrane defect i.e. present both in the central nervous system, cornea, lens, erythrocytes and kidney tubules. Another possibility is that several if not all of the features are secondary to the severe chronic HCl acidosis.

REFERENCES

- 1 Brenes L G, Brenes J N & Hernandez M M. Familial proximal renal tubular acidosis: A distinct clinical entity. *Am J Med* 63: 244, 1977.
- 2 Donckerwolcke R A, van Stekelenburg G J & Tuddens H A. A case of bicarbonate losing renal tubular acidosis with defective carboanhydrase activity. *Arch Dis Child* 45: 769, 1970.
- 3 Cooper R A & Jandl J H. Bile salts and cholesterol in the pathogenesis of target cells in obstructive jaundice. *J Clin Invest* 47: 809, 1968.
- 4 Graziani G, De Vecchi A & Rosti M. Primary proximal renal tubular acidosis: A therapeutic approach and long term follow up. *Helv Paediatr Acta* 31: 47, 1976.
- 5 Mercado A, Slatopolsky E & Klahr S. On the mechanisms responsible for the phosphaturia of bicarbonate administration. *J Clin Invest* 56: 1386, 1975.
- 6 McSherry E & Morris R C Jr. Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis. *J Clin Invest* 61: 509, 1978.
- 7 Morris R C Jr. Renal tubular acidosis: Mechanisms, classification and implications. *N Engl J Med* 281: 1405, 1969.
- 8 Morris R C Jr, Sebastian A & McSherry E. Renal acidosis. *Advances Int* 1: 37, 1972.
- 9 Muldowney F P, Carroll P V, Donohoe J F & Freeman M. Correction of renal bicarbonate wastage by parathyroidectomy. *Q J Med* XL: 478, 1971.
- 10 Narins R C & Goldberg M. Renal tubular acidosis. Pathophysiology, diagnosis and treatment. *DM* 23: 1977.
- 11 Nash M A, Torrado A D, Greiter I, Spitzer A & Edelmann C M Jr. Renal tubular acidosis in infants and children. *J Pediatr* 80: 738, 1977.
- 12 Oetliker O & Rosv E. The influence of extracellular fluid volume on the renal bicarbonate threshold: A study of two children with Lowe's syndrome. *Pediatr Res* 3: 140, 1969.
- 13 Pavone L, Musumeci M, I Voli S, Distefano G & Mollica F. Haematological studies in a case of oculo-cerebrorenal syndrome. *Helv Paediatr Acta* 31: 409, 1976.

3) and Na bicarbonate (18 mmol \times 3) resulted in mean increments of pH from 7.12 to 7.24 and standard bicarbonate from 10 to 13 mmol/l but the growth rate did not improve. A complete correction of the acidosis in our patient B.H. would be impossible by peroral treatment with NaHCO_3 as it probably would require more than 1000 mmoles daily as estimated from the bicarbonate infusion studies.

The beneficial effect of diuretics in proximal RTA is probably due to contraction of the extracellular volume which increases the tubular reabsorptive capacity for bicarbonate (4, 12, 15). However, therapeutic trials in our patient have been unsuccessful.

Clinical characteristics

Bicarbonate wasting of the degree present in this case is rare. To our knowledge the only case similar to our patients is that of Donckerwolcke et al. (2). Their patient, a girl, had some of the features of our patients, i.e. mental and growth retardation. She had also corneal changes but no nystagmus.

Growth failure is regularly present in RTA (6, 10, 20). The growth rate improves markedly when the acidosis is corrected, indicating that the acidosis itself may be causative (6). Intrauterine growth was normal in our patients, whereas growth was retarded in the first couple of years. From about 1–2 years the growth rate has been near normal in B.H. possibly due to adaptation to the chronic acidosis.

Mental retardation has not been regularly found in cases of RTA except in the oculocerebrorenal syndrome of Lowe (10, 12, 20) where it is more severe than in our patients. In various types of chronic metabolic acidosis due to inborn errors of metabolism resulting in accumulation of organic acids, mental retardation is common and probably due to the metabolic block with either accumulation or deficiency of metabolites. In the pure HCl acidosis of our patients no specific metabolic block outside the kidney is obvious although several organs are affected. It seems conceivable that the extremely low pH probably

present since birth might adversely interfere at the biochemical level resulting in disturbed psychomotoric development and function.

The congenital nystagmus present in our patients has not been present in other cases of severe chronic acidosis. The opacities in cornea have been present since the age of 1½ and 5 years in the two brothers and seem to increase with age. Corneal changes although somewhat different (described as band keratopathy) were also present in the patient of Donckerwolcke et al. (2). The vision has deteriorated during the last years in B.H. due to progression of the cataract. At the age of eleven bilateral glaucoma was diagnosed in B.H. Glaucoma has not been reported to develop in RTA except for patients with Lowe's syndrome where it usually is congenital (17, 13, 20).

Rather severe enamel defects were present in the permanent teeth of B.H. According to the dentists it is obvious that these defects originate from the period of enamel development and are not due to exogenous causes after eruption. This is in agreement with the fact that the primary dentition was normal in both patients. Our patients have no rickets and except for moderately retarded bone age skeletal X-ray examinations have been normal.

Biochemical and haematological data

Both our patients had pathologically low PBI and thyroxine values and probably related to this the thyroid gland was very small in O.H. In the girl described by Donckerwolcke et al. (2) PBI was also in the low normal range. Due to low stature and low PBI values O.H. was treated with 1 thyroxine 0.05 mg daily for more than one year without any effect on the growth rate. The patients did not exhibit any clinical signs of hypothyroidism except for reduced height.

The haematological status was usually normal in both boys except for a slight reticulocytosis. $^{51}\text{CrO}_4$ labelling of the erythrocytes of B.H. revealed a mean survival of 20–

71 days against 24 days as the lower normal limit. This is similar to the findings of Pavone et al (13) in a patient with Lowe's syndrome. They suggested an extra erythrocytic factor as being responsible. However, the finding of abnormally increased osmotic resistance of the erythrocytes (Fig. 3) would, in our patients, probably favour a change in some intrinsic property of the erythrocyte membrane itself. This is because incubation of normal erythrocytes in the patients' serum did not result in increased osmotic resistance, neither did incubation of the patients' erythrocytes with normal serum lower the osmotic resistance. In this connection, it is of interest that Morris et al (8) because of the association of familial RTA and elliptocytosis suggest the possibility that the cell membrane of renal tubular cells and erythrocytes share a common protein.

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- 3 Cooper R A & Jandi J H. Bile salts and cholesterol in the pathogenesis of target cells in obstructive jaundice. *J Clin Invest* 47: 809, 1968.
- 4 Graziani G, De Vecchi A & Rosti D. Primary proximal renal tubular acidosis: A therapeutic approach and long term follow up. *Helv Paediatr Acta* 31: 477, 1976.
- 5 Mercado A, Slatopolsky E & Klahr S. On the mechanisms responsible for the phosphaturia of bicarbonate administration. *J Clin Invest* 56: 1386, 1975.
- 6 McSherry E & Morris R C Jr. Attainment and maintenance of normal stature with alkali therapy in infants and children with classic renal tubular acidosis. *J Clin Invest* 61: 509, 1978.
- 7 Morris R C Jr. Renal tubular acidosis: Mechanisms, classification and implications. *N Engl J Med* 281: 1405, 1969.
- 8 Morris R C Jr, Sebastian A & McSherry E. Renal acidosis. *Kidney Int* 13: 1977.
- 9 Muldowney F B, Carroll B V, Donohoe J F & Freaney R. Correction of renal bicarbonate wastage by parathyroidectomy. *Q J Med* XL: 478, 1971.
- 10 Narins R G & Goldberg M. Renal tubular acidosis: Pathophysiology, diagnosis and treatment. *DM* 23: 1977.
- 11 Nash M A, Torrado A D, Greifer I, Spitzer A & Edelmann C M Jr. Renal tubular acidosis in infants and children. *J Pediatr* 80: 738, 1977.
- 12 Oetliker O & Rossi E. The influence of extracellular fluid volume on the renal bicarbonate threshold: A study of two children with Lowe's syndrome. *Pediatr Res* 3: 140, 1969.
- 13 Pavone L, Musumeci S, Li Volti S, Distefano G & Mollica F. Haematological studies in a case of oculocerebrorenal syndrome. *Helv Paediatr Acta* 31: 509, 1976.

- 14 Puschett J H & Zurbach P. Acute effects of parathyroid hormone on proximal bicarbonate transport in the dog. *Kidney Int* 10 501 1976
- 15 Rampini S, Finconi A, Illy R & Prader A. Effect of hydrochlorothiazide on proximal renal tubular acidosis in a patient with idiopathic de Toni Debré Fontana syndrome. *Helv Paediatr Acta* 23 13 1968
- 16 Siddiqui A A & Wilson D R. Primary hyperparathyroidism and proximal renal tubular acidosis. Report of two cases. *Can Med Assoc J* 106 654 1972
- 17 Soriano J R. The renal regulation of acid base balance and the disturbances noted in renal tubular acidosis. *Pediatr Clin North Am* 18 529 1971
- 18 Soriano J R, Boichis H, Stark H & Edelmann C M Jr. Proximal renal tubular acidosis. A defect in bicarbonate reabsorption with normal urinary acidification. *Pediatr Res* 1 81 1967
- 19 Soriano J R, Boichis H & Edelmann C M Jr. Bicarbonate reabsorption and hydrogen ion excretion in children with renal tubular acidosis. *J Pediatr* 71 802 1967
- 20 Soriano J R & Edelmann C M Jr. Renal tubular acidosis. *Annu Rev Med* 20 363 1969

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227 ROAD ACCIDENTS TO CHILDREN

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ABSTRACT Illingworth C M (Accident and Emergency Department Children's Hospital Sheffield England) 227 Road accidents to children *Acta Paediatr Scand* 68 869 1979.—In a 9 month period 2.7 children attended the Accident and Emergency Dept of the Children's Hospital Sheffield after accidents on the road—about 12% of all new attendances 169 were accidents to pedestrians 31 to cyclists and 27 to passengers Of the 169 pedestrian accidents 157 were hit by moving vehicles 72 (45.9%) suffering serious injury with two dead 45% of the pedestrians 22.6% of the cyclists and 11.1% of the passengers were admitted 29.6% of the 227 had severe head injury (concussion with or without a fractured skull) 22% of the 227 had a fracture or fractures 55 children had had previous accidents Compared with 225 previously described skateboard injuries and 200 playground equipment injuries those injured on the roads were much more serious though with fewer fractures 37% of the road accidents were serious compared with 10.7% of those injured on skateboards and 7.5% of the play equipment cases 40.9% of the skateboard injuries but only 7.2% of the road accidents involved fractures but 29.6% of the latter 6.0% of the play equipment injuries and 0.9% of the skateboard injuries involved serious head injury 4.0% of the skateboard injuries 10.0% of the play equipment injuries but 45.0% of the pedestrian road accident cases necessitated admission

KEY WORDS Road accidents children

Accidents are the main cause of death of children aged 1-15 and the cause of a vast amount of morbidity often with much pain and psychological distress and they cause great anxiety to their parents Road accidents are an important cause of morbidity and mortality in children and in the Accident and Emergency Department of the Children's Hospital Sheffield which deals with medical and surgical emergencies road accidents in the period of this study contributed approximately 12% of all new attendances (which total approximately 25 000 per year)

PATIENTS AND METHODS

With the help of a proforma details were recorded of 227 consecutive new attendances at the Accident and Emergency Department of the Children's Hospital on account of accidents on the road paying particular attention to the way in which the accident happened with details from witnesses and the child and to the nature of the injuries The accidents covered a period of 9 months The children

were seen by the Consultant in charge (C M I) or other Accident and Emergency staff which consisted of two part time Registrars and four Senior House Officers The mean age of the children was 7.9 years just over half being age 5 to 9 and a third 10 to 15 169 children were on foot 77 were passengers and 31 were on bicycles 62 of the pedestrians and 93% of the cyclists were boys The severity of the injuries was graded as follows

Grade 1 trivial usually no treatment or follow up necessary

Grade 2 minor lacerations soft tissue injuries or bruises

Grade 3 more serious lacerations undisplaced limb fractures or minimally displaced greenstick fractures and minor head injuries

Grade 4 fractures for which admission was essential seriously displaced fractures that needed manipulation under a general anaesthetic and head injuries with concussion or skull fracture or both

Grade 5 potentially life threatening conditions—for example ruptured viscus or serious head injuries The grading was done by the Consultant (C M I) and was made when the child's treatment was completed or all the facts about the injuries were known The grading was the same as that previously used in a paper on playground equipment injuries (1) and skateboard injuries (2) so that comparisons between the results of accidents could be made

Table 1 Comparison of injuries on skateboards, playground equipment, roads

	Source of Injury					
	Skate board	Playground equipment	Road			Total
			Pedestrian	Passenger	Cycle	
Mean age (years)	11.1	6.3	7.6	8.0	9.4	7.9
Number of cases	225	200	169	27	31	227
Injury grade 1 %	1.8	28.5	0	7.4	0	0.9
Injury grade 2 %	44.0	45.0	21.3	14.8	29.0	21.6
Injury grade 3 %	43.9	19.0	34.9	66.7	48.4	40.5
Injury grade 4 %	10.7	6.5	33.7	3.7	16.1	17.8
Injury grade 5 %	0	1.0	8.9	3.7	6.5	7.9
Died %	0	0	1.2	3.7	0	1.3
Fractures %	40.9	26.5	26.0	7.4	16.1	22.0
Concussion with or without skull fracture %	0.9	6.0	33.1	11.1	22.6	9.6
Soft tissue injuries alone %	33.8	22.5	23.7	19.4	22.2	22.9
Admitted %	4.0	10.0	45.0	11.1	22.6	37.9
Injuries above neck %	15.6	46.0	67.4	70.4	77.4	69.7
X-rays taken %	68.4	59.5	68.6	33.3	67.9	67.3

Head injuries were subdivided into three groups—severe denoting concussion or fractured skull or both; minor denoting significant haematoma or laceration over the skull; and other injuries above the neck—abrasions and less severe lacerations, bruises, nose bleeds, etc.

Of the 227 children, 20 came from outside the city boundary. Approximately one third were going to or from school, and just over a half were on holiday in the week ends or outside school hours, but figures for this are not accurate. At least 6 were known to come from problem families.

RESULTS

Table 1 shows the grading of the injuries: 0.9% in grade 1, 21.6% in grade 2, 40.5% in grade 3, 27.8% in grade 4, and 7.9% in grade 5, with 3 deaths (1.3% of the total). It will be seen that 43.8% of the pedestrians, 22.6% of the cyclists, and 11.1% of the passengers had the most serious accidents—grades 4, 5 or

Table 2 Road accidents: details

	Pedestrian (n=169) (%)	Passenger (n=27) (%)	Cycle (n=31) (%)	Total 227 (%)
Head injury: Severe				
Solely	7.1	7.4	6.5	7.5
Fractured skull	3.6	3.7	6.5	4.0
Other fracture	9.5	0	0	7.1
Other soft tissue injury	13.0	0	9.7	11.0
Head injury: Minor				
Solely	10.1	30.0	9.7	12.3
Fracture elsewhere	2.4	0	6.5	2.7
Other soft tissue injury	14.8	3.7	19.4	14.1
Other injury above neck: bruises, lacerations, abrasions				
Solely	0.6	22.2	9.7	4.4
Fracture elsewhere	1.2	0	0	0.1
Other soft tissue injury	5.3	3.7	9.7	5.7
Fractures other than above	8.9	7.4	3.2	7.9
Soft tissue injury other than above	23.7	22.2	19.4	22.9

death. But of the 169 pedestrians 12 had merely fallen on the road, 9 of them on ice, and had not been hit by a vehicle; only two of those had worse than a grade 2 or 3 injury. Of the 157 hit by a moving vehicle, 72 (45.9%) had a grade 4 or 5 injury and two died. 37.9% of the 227 were admitted. 45.0% of the pedestrians, 22.6% of the cyclists and 11.1% of the passengers.

Approximately two-thirds of the pedestrians and three quarters of the cyclists and passengers had injuries above the neck. 29.6% of the 227 had a severe head injury; the figure for pedestrians was 33.2%, for passengers 11.1% and for cyclists 22.7%. 4% of the total had a fractured skull, and 7.1% of those with a severe head injury had fractures elsewhere. Those with severe head injury were admitted, but 27 of the 66 with minor head injuries were allowed home after a period of observation in the Department, and with the usual head injury instructions. Table 2 shows that of the 227 children, 22.0% had fractures (skull or elsewhere) and 22.9% had soft tissue injuries other than those above the neck. Several had multiple fractures. Four had injury to the kidney, with haematuria, and 2 had rupture of the spleen. 7 others had abdominal injury and one had laceration of the anus and rectal mucosa.

X-rays were taken in approximately two-thirds of the children, but in 39% of the head injuries an X-ray of the skull was not thought necessary.

Fifty-five of the children had had previous accidents (24.2%); their mean age was 8.2 years. Two-thirds were boys. Twenty of the 55 had had two or more accidents and 8 had had four or more. Five came from known problem families.

Under *Causes of Accidents* I have described the ways in which the accidents happened. In many cases, owing to retrograde amnesia, it was impossible to obtain an account from the child, but inaccurate as it must be, we tried our best to determine how the accidents happened. Several children admitted that they had not looked before crossing the

road, or that they looked one way only. In at least 22 cases the child's vision was obscured by stationary vehicles—in 19 cases the child passed between buses and cars and was then hit by a vehicle. Eighteen said that they had not seen an approaching vehicle, in two cases it was foggy, but in three cases the car came round a corner and could not be seen approaching, and in one case a car performed an unexpected U-turn. Eight children were hit when running across the road to friends at the other side. At least 12 were playing on the road, hiding, chasing or being chased. Six were hit on a pedestrian crossing. Two were deaf. It is impossible to say how many accidents were due to the child, with his inexperience, underestimating the speed of a car. One boy said he was crossing the road when this raving idiot came and knocked me half a mile up the road!

As for passengers, 3 fell out of a moving car on a corner, and 2 were injured in a stationary car when the car was hit in the rear by another vehicle. Three of the accidents involved babies in prams. In one case a pram pushed by a 15-year-old aunt was hit by a car. In another, a father pushing the pram misjudged traffic lights; the pram was hit and the baby killed.

The cycle accidents were due to the usual causes—collisions at a road junction, hitting an obstruction or skidding. In one case a wheel came off; in another the boy braked too violently and went over the handlebars.

Causes of Accidents

Pedestrian (total 169)

Falls on road only, not hit by vehicle: 17 (9 fell on ice, one fell down a bank onto moving car); all others (157) hit by vehicle.
Hit by vehicle, no other information: 51.
Admitted not looking: 13.
Admitted not seeing car: 18 (? in fog, car came round corner; 3 car performing U-turn; 1).
Friends or mother on opposite side, child running to them: 8.
Admitted running across road: 13 (? hit by police car, one fell off parked lorry, ran across road and hit, one fell when running across and was run over).
Playing on road, hiding behind car, chasing or being chased: 17.

Vision obscured 22 (19 crossing from between buses or cars, 3 from behind ice cream van)
 Pedestrian or supervised crossing, 6 (one car or bus stopping, another vehicle failing to stop, one child was crossing with mother, both hit)
 Pedestrian crossing had been moved, child crossed, hit
 Deaf 2
 Run behind backing car, 2, in front of parked car when it moved 2
 Jumped off wall onto moving car, 1
 Run down bank, could not stop, hit by car
 Hesitation, children run into road, car stopped, car restarted, children run back, hit 2

Cyclist

Hit by car (car came from a turning, 1 cyclist failed to give way at junction, 2 cyclist crossed road without looking, 1)
 Cycling across pedestrian crossing, hit
 Hit by skidding car, 1, by reversing car, 1
 Collided with another cycle, 1
 Wheel came off, 1
 Braked too violently, went over handlebars, 1
 Hit stone wall, curb, 6
 Just fell off, 3
 Skidded, 2

Passenger

Car collision, 16
 Fell out of moving car, 3
 Fell off milk trolley, 1, off motor cycle pillion, 1, off bus, 1
 Sitting in stationary car, car hit in rear, 2

Comparison with Two Previous Studies

Table 1 compares the present data with those of two previous studies, one of 225 skateboard injuries and the other of 200 playground equipment injuries (1, 2). The mean age of the skateboarders was higher (11.1 years) than that in the other two studies. It will be seen that 45.8% of the skateboard injuries and 73.5% of the play injuries fall into grades 1 and 2 compared with 22.5% of the road accidents and only 10.7% of the skateboard accidents and 7.5% of the play injuries fall into grades 4 and 5, the most serious compared with 37.0% of the road accidents (three of which were fatal). The percentage of fractures (40.9%) was almost double that in the road accident cases but skateboard accidents led to serious head injuries in 0.9%, play equipment in 6.0% and road accidents in 29.6%. Only 4.0% of the skateboard accidents led to admis-

sion, 10.0% of the play injuries but 37.9% of the road accidents (including 45.0% of the pedestrian injuries).

DISCUSSION

The serious problem of accidents to children on the roads is indicated by the fact that 66 of the 227 children suffered concussion with or without a fractured skull, that 63 children (57 of them pedestrians) had grade 4 accidents, 18 (grade 5) suffered life threatening injury and three died. More than two thirds of the children had injuries above the neck.

These are not new problems but the publicity given to them is minute compared with that given to accidents on skateboards and playground equipment—which have less than half as high a percentage of most serious injuries. Accidents on skateboards resulted in a much higher number of fractures (40.9%) than accidents on the roads (22.0%) but the majority of the skateboard fractures were comparatively trivial compared with the fractures resulting from road accidents.

It is easy to blame children for inviting injury by playing on the road—but they may have no other place. They may misjudge the speed of traffic and many of them are not old or mature enough to be able to do so accurately. Many adults are not good at this either! The children's small size makes it impossible for them to see over parked cars so that they often emerge from a tunnel into fast moving traffic. Young children are easily distracted especially when they are with other children so that if they see someone or something interesting on the other side of the road they may rush across and forget completely that there may be dangers in the way. Running home after school they may be tired and almost certainly hungry, both will reduce their powers of concentration and alertness.

Parents may be to blame when they leave young children in potentially dangerous situations which are too complex for them to deal with at their stage of development but chil-

dren have to be allowed to grow up and acquire independence. Over protection has the opposite of the effect desired leading to accident proneness. It is easy to blame parents but even the most careful parents have to some degree to take calculated risks with their children. Children must have some experience of traffic and exposure to traffic in order to become competent to deal with it. Many children have the sort of personality and home background which makes them more likely to have accidents and some children have physical handicaps such as deafness which increase the risk.

As for drivers we cannot say how many of the accidents were due to alcohol or drugs we lag behind other countries in the prevention of driving under the influence of alcohol.

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REFERENCES

- 1 Illingworth C, Brennan P, Jay A, Al Rawi F & Collick M. 200 injuries caused by playground equipment. *Br Med J* 11: 337, 1975.
- 2 Illingworth C, Jay A, Noble M & Collick M. 255 Skateboard injuries in children. *Clin Pediatr* 17: 781, 1978.

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Pedestrian crossing had been moved child crossed hit
 Death 2

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Braked too violently went over handlebars 1

Hit stone wall curb 6

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Sitting in stationary car car hit in rear 2

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COMPUTED TOMOGRAPHIC FINDINGS OF THE BRAIN IN CHILDREN WITH ACUTE LYMPHOCTIC LEUKEMIA AFTER CENTRAL NERVOUS SYSTEM PROPHYLAXIS WITHOUT CRANIAL IRRADIATION

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ABSTRACT Kolmannskog S, Moe P J and Anke I M (Department of Paediatrics University of Tromsø Tromsø Department of Paediatrics University of Trondheim Trondheim and Department of Radiology University of Tromsø Tromsø Norway) Computed tomographic findings of the brain in children with acute lymphocytic leukemia after central nervous system prophylaxis without cranial irradiation. *Acta Paediatr Scand* 68 875 1979.—Nineteen children in primary remission of acute lymphocytic leukemia (ALL) were investigated by computed tomographic (CT) scans of the brain 2 to 64 (mean 19) months after the central nervous system (CNS) prophylaxis was finished. The CNS prophylaxis consisted of high dose Methotrexate (HDM) intravenously combined with 6-8 doses of Methotrexate intrathecally. Two children received only Methotrexate intrathecally as CNS prophylaxis. In addition three children with ALL who had CNS leukemia were investigated by CT scans of the brain. Only one abnormal CT scan was found among the nineteen asymptomatic children and one of the three patients with CNS relapse had slightly dilated subarachnoidal spaces. These results compared with other reports in literature in which the CNS prophylaxis has consisted of intrathecal Methotrexate and cranial irradiation suggest that there are fewer abnormal CT findings of the brain in patients not receiving cranial irradiation as part of CNS prophylaxis.

KEY WORDS Acute lymphocytic leukemia CNS prophylaxis CT findings

Central nervous system (CNS) involvement as a usual event in the natural history of acute lymphocytic leukemia (ALL) (5-11) is one of the most important new steps in the treatment of children with ALL. has therefore been the introduction of CNS prophylaxis (1). Intrathecal chemotherapy combined with cranial irradiation (usually 2400 rads) has been the most commonly used method. However several reports have shown the neurotoxicity of this combination (6, 7, 9, 10, 15, 18-21). Even in asymptomatic children with ALL 50-100% have shown abnormal computed tomographic (CT) findings (2, 17). Lowered intellectual abilities among these patients has been demonstrated (3).

CNS leukemia may also effectively be prevented by using an adequate regimen of Methotrexate intrathecally (8, 11). This method has been further improved by a combina-

tion of Methotrexate intrathecally and high dose Methotrexate (HDM) intravenously as part of a sanctuary phase. This programme is now used on a national basis in Norway (12, 13).

The purpose of this investigation has been to study the CT findings in children with ALL after CNS prophylaxis without cranial irradiation particularly in cases receiving three courses of HDM. CT scanners have just been obtained in three cities in Norway. It has therefore not been possible to perform CT studies on all patients in Norway receiving HDM.

PATIENTS AND METHODS

A total of nineteen children from 3 to 14 (mean 8) years in primary remission of ALL were investigated by CT scans of the brain 2 to 64 (mean 19) months after the CNS prophylaxis was finished. Three children from 6 to 11 (mean 10) years with ALL and CNS leukemia (1 to 4

The question whether Methotrexate used alone will give adequate CNS prophylaxis has been discussed. Moe et al. have only observed 2 cases of CNS leukemia among 34 patients receiving Methotrexate intrathecally as the only type of CNS prophylaxis in the period 1971-1975 (11). Similar low incidence of CNS leukemia has been demonstrated by Hagbin et al. (8). Only two of the first 69 children in Norway receiving HDM during their first remission have so far developed CNS leukemia [13].

Because of the high prevalence of abnormal CT findings in children receiving both Methotrexate intrathecally and cranial irradiation as CNS prophylaxis, it has been suggested that alternative ways of giving CNS prophylaxis should be considered (17). We have found few side effects from the CNS after CNS prophylaxis consisting of Methotrexate intrathecally and HDM, and the results so far seem to be encouraging [13].

REFERENCES

1. Aur R J A, Hustu H O, Verzosa M S, Wood A & Simone J V. Comparison of two methods of preventing central nervous system leukemia. *Blood* 49: 149 1973.
2. Casteels Van Daele M, Van de Casseye W, Casier P, Eeckels R, Palmers Y & Crolla D. CT scans of the brain in children with acute lymphocytic leukemia after prophylactic treatment of the central nervous system. In *Proceedings of the 10th Meeting of the International Society of Paediatric Oncology, Buenos Aires, September 1978*.
3. Eiser C. Intellectual abilities among survivors of childhood leukemia as a function of CNS irradiation. *Arch Dis Child* 53: 391 1978.
4. Enzmann D R & Lane B. Cranial computed tomography findings in anorexia nervosa. *J Comput Assist Tomogr* 1: 410 1977.
5. Evans A H, Gilbert E H & Zandstra R. The increasing incidence of central nervous system leukemia in children. *Cancer* 26: 404 1970.
6. Flament Durand J, Kettelant Balasse P, Maurin R, Regnier R & Spehl M. Intracerebral calcifications appearing during the course of acute lymphocytic leukemia treated with Methotrexate and X rays. *Cancer* 35: 319 1975.
7. Giralt M, Gil J L, Banderas F, Oliveros A, Gomez Pereda R, Pardo J, Martinez Ibanez F & Racho A. Intracerebral calcifications in childhood lymphoblastic leukemia. *Acta Haematol* 59: 193 1978.
8. Hagbin M, Tan C T C, Clarkson B D, Miké V, Burchenal J H & Murphy M L. Treatment of acute lymphoblastic leukemia in children with prophylactic intrathecal methotrexate and intensive systemic chemotherapy. *Cancer Research* 35: 807 1975.
9. Kay H E M, Anapton P J, O'Sullivan J P, Wells D G, Harris R F, Innes E M, Stuart J, Schwartz F C M & Thompson E N. Encephalopathy in acute leukemia associated with methotrexate therapy. *Arch Dis Child* 47: 344 1972.
10. McIntosh S, Fisher D B, Rothman S G, Rosenfield N, Lobel J E & O'Brien R T. Intracranial calcifications in childhood leukemia. *J Pediatr* 91: 909 1977.
11. Moe P J & Finne P H. Intrakranielle leukemiske infiltrater hos barn. *Tidsskr Nor Lægeforen* 95: 874 1975.
12. Moe P J & Seip M. High dose methotrexate in acute lymphocytic leukemia in childhood. *Acta Paediatr Scand* 67: 765 1978.
13. Moe P J. Høy dose metotrexat til barn med akutt lymfatisk leukemi. *Tidsskr Nor Lægeforen* 99: 778 1979.
14. Ochs J J, Berger P E, Brecher M L, Sinks L F & Freeman A I. Computed tomography (CT) scans in children with acute lymphocytic leukemia (ALL) following CNS prophylaxis without radiotherapy. *Proc ASCO* 19: 391 1978.
15. Oliff A, Bleyer W A & Poplack D G. Acute encephalopathy after irradiation of cranial irradiation for meningeal leukemia. *Lancet* 1: 13 1978.
16. Pedersen H, Gyldensted M & Gyldensted C. Mensuration of the normal ventricular system and supratentorial subarchnoidal space in children with computed tomography. To be published.
17. Peylan Ramu N, Poplack D G, Pizzo P A, Adornato H T & Th-Chiro G. Abnormal CT scans of the brain in asymptomatic children with acute lymphocytic leukemia after prophylactic treatment of the central nervous system with radiation and intrathecal chemotherapy. *Engl J Med* 298: 815 1978.
18. Pochedly C. Neurotoxicity due to CNS therapy for leukemia. *Med Pediatr Oncol* 3: 101 1977.
19. Price R A & Jamieson B A. The central nervous system in childhood leukemia. *Cancer* 35: 306 1975.
20. Rubinstein L J, Herman M M, Long T F & Wilbur J R. Disseminated necrotizing leukoencephalopathy: A complication of treated central nervous system leukemia and lymphoma. *Cancer* 35: 791 1975.
21. Young L W. Intracerebral calcifications in treated leukemia in a child. *Am J Dis Child* 131: 1784 1977.

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CNS relapses) were also investigated by CT scans of the brain 1 to 62 (mean 27) months after the CNS leukemia was diagnosed.

The induction phase treatment consisted of vincristine weekly and prednisone or dexamethasone daily followed by a ten day course of L-asparaginase. When remission had been achieved three intravenous courses of HDM (500 mg/m²) were given at 3 weekly intervals. Maintenance therapy was then started with daily oral 6 mercaptopurine and weekly oral Methotrexate and pulses of steroid/vincristine (12).

CNS prophylaxis during the last 3½ years has consisted of three HDM courses intravenously and 6-8 doses of Methotrexate (12 mg/m²) intrathecally. Two patients received only Methotrexate intrathecally as CNS prophylaxis. None of the children has got cranial irradiation as part of the CNS prophylaxis.

The three children with CNS leukemia have not received CNS prophylaxis during the induction phase. After their CNS relapses were diagnosed they have received Methotrexate intrathecally every month after new remission was achieved. They have been given from 5 to 60 doses of Methotrexate intrathecally at the time CT scans were performed.

The CT investigation of 16 children were performed with a Delta Scanner 501S and six children were investigated with an FMI Scanner 5005.

Evaluation of the CT scans was done by a neurologist (I. M. A.). The measurements of normal ventricular system and subarachnoidal spaces in children as described by Enzmann & Lane (4) and Pedersen et al (16) were used.

RESULTS

Of the nineteen children in primary remission of ALL, an abnormal CT scan was only found in a single case: a four year old girl. Four days after the sixth intrathecal Methotrexate injection and five days after her second course of HDM intravenously she had convulsions and lost consciousness. She developed a left sided hemiplegia and electroencephalographic abnormalities of her right hemisphere. The CT scan eight months later showed a small lesion with low and high density and without contrast enhancement in the region of the insula. One year later this abnormality could hardly be seen. She has later recovered almost completely from her hemiplegia and she has no signs of intellectual disturbances.

None of the three children with CNS relapses had enlarged ventricles. Even a girl with four CNS relapses and who had received 60 injections of Methotrexate intrathecally and two courses of HDM showed normal CT find-

ings. Only one of them had slightly dilated subarachnoidal spaces.

DISCUSSION

Different types of abnormalities have been described in patients who have received CNS prophylaxis consisting of cranial irradiation and intrathecal Methotrexate or cytosine arabinoside: ventricular dilatation, widening of the subarachnoidal spaces, areas of decreased attenuation coefficient and intracerebral calcifications (17). There was only demonstrated abnormal CT scan in one of the nineteen children in primary remission of ALL. Probably this abnormality was due to Methotrexate medication. Paresis following intrathecal instillation of Methotrexate has been described earlier (18).

Our number of CT studied cases who have received three courses of HDM are so far small. Ochs et al (14) have however demonstrated pathological CT findings in only one of 35 cases receiving a similar type of combined CNS prophylaxis. These two independent studies strongly suggest that CT abnormalities are rare in children receiving this type of CNS prophylaxis without cranial irradiation. However, it remains to be shown that the CT abnormalities will be associated with signs of brain damage in children, although impairment of intellectual function has already been demonstrated after some years in a large proportion of children receiving irradiation to the CNS combined with Methotrexate intrathecally, while no abnormality was found in a group receiving only Methotrexate as prophylaxis (3).

Observation time is too short for the demonstration of possible late effects of HDM on intellectual functions. It is reassuring, however, that almost all such children seem to have normal CT scans when studied at least two months after CNS prophylaxis was finished. There is a need for larger series with CT scans and later follow up to substantiate this.

PHYSICAL HEALTH SCREENING OF SCHOOL CHILDREN

Extended Health Care Responsibilities for School nurses

R KORNFALT B JÖNSSON and I ROSLUND

From the Dalby Community Health Research Centre Dalby Sweden and the Department of Paediatrics University Hospital Lund Sweden

ABSTRACT Kornfalt R, Jonsson B and Roslund I (Dalby Community Research Centre Dalby and Department of Paediatrics University Hospital Lund Sweden) Physical health screening of school-children. Extended health care responsibilities for school nurses. *Acta Paediatr Scand* 68 879-1979. All 410 ten and twelve year old children of a school district underwent two repeated physical examinations within the school services: the first by the school nurse, the second by the school doctor. The aim was to compare their assessments to see if physical class examinations could be delegated to the nurse in future in order to release doctor's time. More than half of the children were found to have slight deviations from normal, most common of the spine and in the skin. The nurse detected many more deviations than the doctor but their assessments showed good agreement concerning functionally important deviations. Newly detected functionally important deviations were noted in 8 children (2%).

The routine physical examination could perfectly well be delegated to the school nurse who has the necessary prerequisites to take this responsibility and screen out those children in need of a doctor's assessment. In this study 20%. She would release valuable time for the doctor who could then apply himself to the real health problems of the children of today: chronic diseases, behavioural and school problems, many of which frequently are concerns beyond the boundaries of traditional medical care.

KEY WORDS School health, physical health-screening, school nurse, nurse practitioners, primary care.

Changing health needs and changing concepts of health have made a re-evaluation of School Health Programmes necessary. Infectious diseases and nutritional defects are no longer major health problems. Today accidents, handicaps, chronic diseases and emotional disturbances are more important. The greatest problems of the School Health Service today are behavioural defects, inadequate functioning in school and adjustment problems in adolescence (13).

The School Health Service, which has the major responsibility for the health of school-aged children, has very often insufficient resources for today's greater need to work within all areas of the children's lives (2, 3, 6, 14, 20).

The Swedish School Health Service was or-

ganized in its present form (5) in 1944. The aim is to follow up the growth and development of school children, to retain and improve their mental and physical health, and to influence them to form healthy habits. The most important aspect within the Service is preventive in character. The programme includes periodic physical examinations, vision and auditory screening, and a vaccination programme. The School Health staff consists of a physician, visiting school once a week, and a nurse, usually fulltime. The physician makes traditional physical examinations in the first, fourth, seventh and ninth classes of the elementary school, i.e. at 7, 10, 14 and 16 years of age. It has recently been suggested that the last two examinations should be exchanged for one only in the eighth class (24).

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Table 1 Classification of health problems

Health problem	Group 0 Healthy child	Group 1 Slight deviation without importance	Group 2 Moderate deviation treatment indicated	Group 3 Definitely handicapping disorders
Somatic development		Weight or height $> \pm 2$ S D	Obesity $> +3$ S D	Chronic disease e.g. severe asthma, diabetes, uncontrolled epilepsy etc.
Skin abnormality		Warts, warts, minor allergy, extensive acne	Moderate allergic eczema, Mycosis	Ichthyosis, severe allergic eczema
Spine deformity		Bad posture, static scoliosis, kyphosis	Structural scoliosis $> 10^\circ$ deviation (Cobb)	Structural scoliosis with brace treatment or operation
Joint problems		Knock knees	Arthritis, tendinitis, Mb Schlatter	Rheumatoid arthritis
Feet problems		Pes planus		
Abnormality in motor development		Slight disturbances in fine and gross movements	Minor brain dysfunction (MBD) e.g. disturbances in fine and gross movements combined with perception difficulties	Cerebral palsy or other severe motor handicap

The value of having physicians performing the periodic routine physical examinations has been questioned. Several evaluations of the yield of these examinations have been discouraging (2, 15, 17, 18, 30, 31).

The Swedish school physician, however, still spends more than half of his time on routine physical examinations (19) and has seldom time for the children's individual social or emotional problems. The working conditions in the School Health Service are by many doctors conceived as unsatisfactory (27).

The school nurse is the main resource of the school health team. She is in close contact with all the children in her area as well as with their teachers. She comes to know the children well and learns of their problems when they come to her for minor ailments and accidents or to be weighed and measured, vaccinated or have their vision tested. The children find a refuge with her when they feel bad whatever the cause. Her assessment of each child's health should therefore be at least as reliable as the doctor's. Studies based on registrations of consultations with the Swedish school nurse show that she can handle 80% of the problems on her own without help from a doctor (16, 19). It seems probable that she

has the prerequisites to make correct assessments of the children's physical health also in routine examinations. In this way valuable time would be released for the doctor who could then devote more interest to medical as well as psychosocial problems.

In this study the periodic routine physical examinations were delegated to the school nurses. Their assessments were compared with the doctors who examined the same children with a traditional examination. The authors have also studied the frequency of social and behavioural problems in the children based on parents', children's and teachers' interviews. The results will be presented in a separate paper.

MATERIALS AND METHODS

The material comprises all 10-year-old ($n=723$) and all 13-year-old ($n=187$) children from the primary school of the Dilby area in southern Sweden.

Details of the physical examinations of 10-year-old children have previously been reported (15). All the children were examined by the permanent school health team of the district: 2 nurses and 1 physician. The physician (R. K.) is a trained paediatrician with 3 years' experience of school health work and a special interest in social paediatrics.

The school nurses had 8 and 3 years of experience of school health work in the district, respectively. Prior to

Table 2 *Functionally important health problems in 410 10 to 12 year old children*

	10-year old <i>n</i> = 223 (%)	12-year old <i>n</i> = 187 (%)	Sum <i>n</i> = 410 (%)
History and physical examination	11.7	15.1	13.4
Newly detected	0.9	2.1	1.5
Previously known	10.8	13.0	11.9
Vision examination	11.7	13.9	12.8
Newly detected	7	10	18
Previously known	9.0	12.9	10.9
Auditory examination	7	3.0	2.8
Newly detected	0.9	0.6	0.7
Previously known	1.8	2.4	2.1
Total	26.2	30.0	28.0
Newly detected	4.5	4.0	4.2
Previously known	21.7	26.0	23.8

the examination the nurses had repeated training sessions with demonstrations by the doctor. A trained physiotherapist demonstrated how to make an examination of the spine. In addition the nurses had access to a written manual of instructions.

Each nurse examined about 100 children once and the doctor examined all of them once. First the children were examined by the nurses and about two weeks later by the doctor who at that time was ignorant of the nurses' results. A standardized and structured schedule including a manual was used by all examiners.

The following aspects of the physical examinations were studied and compared: somatic and motor development, skin, spine, joints and feet. The methods of classifying deviations are shown in Table 1.

In addition the doctor's physical examination included auscultation of the heart, measurement of blood pressure, palpation of the thyroid gland and lymphnodes of the abdomen and in boys of the genital organs.

The nurses' physical examination included a vision and an auditory screening. The methods are described elsewhere (15) and the results are only briefly reported here.

Additional data of the children's health problems were registered with the help of written questionnaires to the parents and from interviews with their teachers. These results will be published separately.

Weight and height were plotted on standard curves for Swedish children (25). Signs of scoliosis were specifically looked for in the spine examination. The children were observed from the front side and back while standing erect and bending forward, the spine being flexed to approximately 60° (4, 8).

Shoulder height asymmetry or prominence of the rib cage were noted. Static scoliosis was excluded by comparison of unequal leg length with a plate under the shorter leg.

Evaluation of the motor development was made by testing the coordination of fine and gross movements. Each

child was observed when walking normally, tiptoeing, walking on his heels and on the lateral borders of his feet (Fog's test) (9) and when jumping on one leg. Results of the Fog's test revealing pronounced or unilateral supination, pronation and extension or excessive grimacing were scored as abnormalities. The testing of involuntary movements described by Prechtl (26) but modified (15) was used. Fine movements were observed when the child in rapid succession and in proper order opposed the thumb to the fingers of the same hand, threaded a string, drew a circle, rhomb and square. Deviations in any of the described exercises classified the child as clumsy (Table 1).

The health problems or deviations from normal were classified as to their severity or functional importance in groups 1, 2 and 3 (Table 1) according to a method introduced by Kohler (17).

Deviations of groups 2 and 3 were classified as significant or functionally important and the slight deviations of group 1 as insignificant or unimportant. Health problems that had already been detected and were under observation or treatment before the actual investigation were defined as previously known.

The nurses divided their children into two groups according to their need for a doctor's examination: 1. Referral, 2. No referral. The time needed to examine each child was recorded.

RESULTS

An analysis of all physical health problems including visual and auditory defects of the 10-year-old children have been published earlier (15). The health problems of the 12-year-old children were found to be very much the same (Table 2).

All deviations disclosed in the physical examinations are presented in Figs. 1 and 2. More than half of the children were found to have slight deviations from normal. The nurses registered 104 more deviations than the doctor. The doctor detected 35 deviations not observed by the nurses. The nurses classified 29 (11%) of their deviations as functionally important, the doctor 23 (14%) of his. The doctor did not classify 8 of the nurses' findings as significant, 2 deviations in somatic development and 6 cases of suspected scoliosis. The doctor noted 2 significant disorders not observed by one of the nurses: Morbus Schlatter in a 12-year-old boy and allergic eczema in a 10-year-old girl. It is possible that these children had less symptoms when the nurse made her examination three weeks earlier.

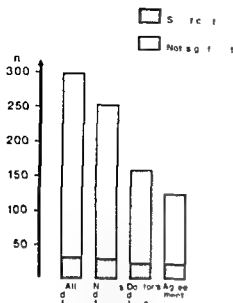


Fig. 1 Deviations detected in physical examination of 10 to 12 year old children

Overweight (weight/height $\geq +2$ SD) was apparent in 5% of all the children and spine deformities in more than 10%. Of all children 15% had significant structural scoliosis. Slight disturbances in motor development were noted in 8% of the children less frequent in the 12 year-olds (5%) than in the 10 year-olds (10%).

Altogether 8 significant disorders were newly detected by the doctor (Table 3). Half

Table 4 A test for accuracy of the nurse's physical examination as a screening examination

Nurse	Doctor		Total
	Detected significant health problems	Not detected significant health problems	
Detected significant health problems	21	8	29
Not detected significant health problems	2	379	381
Total	23	387	410

Sensitivity $(21 \times 100)/29 = 91\%$ Specificity $(379 \times 100)/387 = 98\%$ Positive predictive value $(21 \times 100)/29 = 72\%$ Youden Index $(\text{sensitivity} + \text{specificity} - 100) = (91 + 98)/100 = 89\%$

of them were structural scoliosis later confirmed by an orthopaedic specialist and put under close observation.

If the nurse's examination is regarded as a screening examination and the doctor's evaluation of significant health problems is considered as a diagnostic examination, the accuracy of the physical screening could be calculated according to Table 4. The specificity would be 98%, the sensitivity 91%, the posi-

Table 3 Functionally important health problems observed by the doctor in the physical examination

Diagnoses	Previously known (n)	Newly discovered (n)	Total (n)
Obesity	4	0	4
Asthma	1	0	1
Allergic rhinoconj	0	1	1
Aplasia of fibula	1	0	1
Ichthyosis	1	0	1
Allergic eczema	4	0	4
Foot mycosis	0	1	1
Scabies	1	1	2
Structural scoliosis	2	4	6
Mb Schlatter	1	1	2
Flat feet	1	0	1
Total	15	8	23

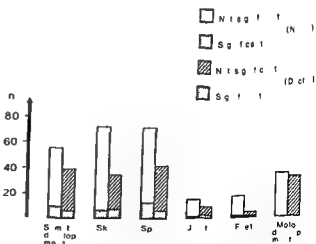


Fig. 2 Deviations from normal diagnosed by the nurse and the doctor in the physical screening of 410 10 to 12 year old children

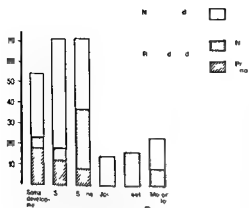


Fig 3 Referrals to the school physician by the nurses

ive predictive value 72% and the Youden index 89% (10)

The traditional auscultation and palpation examination was also made on the children by the school physician. Two heart murmurs were noted both previously known and under treatment. In addition two boys with phimosis were newly detected and referred for surgery.

The nurses considered that their screening was sufficient as a physical appraisal in 80% of the children. In the other 20% they considered that doctor's consultation was needed (Fig 3). Half of these children had health problems previously known and in need of a follow-up. In the other half the nurses suspected deviations mostly in growth and development and of the spine.

The school physician could not find any abnormality in half of the cases which were referred to her (Fig 4). The nurses and the physician spent an average of 5 min on each child. Since the nurse measured weight and height and performed a vision screening at the same session the added examination did not take up much extra time. Thus if the nurse is made responsible for the physical screening examination and takes care of 80% of the problems on her own the doctor would approximately save 80% of the time he usually spends on class-examinations.

DISCUSSION

The purpose of physical examinations of school children is to reveal functionally important defects in need of treatment and to detect conditions where appropriate intervention can minimize or prevent functional impairment. Deviations in growth and development, spine deformities and allergic diseases are examples of health problems which often are asymptomatic or neglected in prepubertal age if they are not specifically looked for in health controls. Thanks to an efficient preschool health programme in Sweden, handicaps and chronic disorders such as neurological diseases and congenital heart diseases are fully controlled. In the present investigation 8 functionally important health problems were detected by simply looking at the child while the traditional doctor's examination with auscultation and palpation revealed only 2 cases of phimosis which should have been discovered at the school entrance. This study indicates that examinations of the children by a nurse only would be quite sufficient as a screening with high sensitivity and specificity regarding functionally important deviations.

The role and the skill of the Swedish school nurses seem to be underestimated. Their medical training is solid and comprehensive and qualifies them to take advanced medical responsibilities in health surveillance and primary

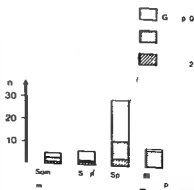


Fig 4 The school physician's classification of newly detected health problems referred by the school nurses

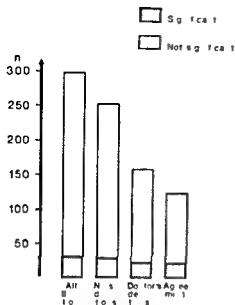


Fig. 1 Deviations detected in physical examination of 10 to 12 year old children

Overweight (weight/height $\geq +2$ S D) was apparent in 5% of all the children and spine deformities in more than 10%. Of all children 1.5% had significant structural scoliosis. Slight disturbances in motor development were noted in 8% of the children less frequent in the 12 year-olds (5%) than in the 10 year olds (10%).

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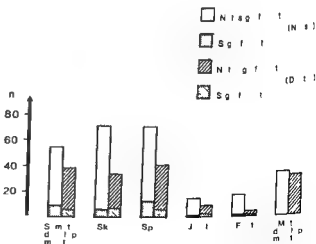


Fig. 2 Deviations from normal diagnosed by the nurse and the doctor in the physical screening of 410 10 to 17 year old children

- 11 Grant M W W Fearnow R G Herbertson C L M & Henderson A L Health screening in school age children The physician and paramedical personnel *Am J Dis Child* 125 5 1973
- 12 Greenfield S Komaroff L Pass T M Anderson H & Nessim S Efficiency and cost of primary care by nurses and physician assistants *N Engl J Med* 298 305 1978
- 13 Haggerty R J Roghmann K J & Pless I H *Child health and the community* Wiley New York 1975
- 14 Helve A Skolhalsvården en del av folkhälsoarbetet i Finland *Nord Med* 91 85 1976
- 15 Kornfalt R & Kohler L Physical health of ten year-old children An epidemiological study of school children and a follow up of previous health care *Acta Paediatr Scand* 67 481 1978
- 16 Kornfalt R Malmberg M & Roslund I Report of a school health team in an elementary school In preparation
- 17 Kohler L Physical health of 7 year old children An epidemiological study of school entrants and a comparison with their preschool health *Acta Paediatr Scand* 66 797 1977
- 18 Kohler L Physical mass examinations in the school health service *Acta Paediatr Scand* 66 307 1977
- 19 Kohler L & Andersson I Case report of a Swedish school health clinic *Acta Paediatr Scand* 68 Suppl 775 1979
- 20 *Problems of children of school age (10-13 years)* Report on a working group WHO Copenhagen 1976
- 21 Sells C J & May E A Scoliosis screening *Am J Nurs* 74 60 1974
- 22 Silver H K Iggo J B & McAtee P R The school nurse practitioner Providing improved health care to children *Pediatrics* 58 580 1976
- 23 Silver H K Ford L C & Day L R The pediatric nurse practitioner programme *JAMA* 240 798 1968
- 24 *Skolhälsovård* Betänkande av 1974 års skolhälsovårdsutredning SOU 1976 46
- 25 Taranger J The somatic development of children in a Swedish urban community A prospective longitudinal study *Acta Paediatr Scand* Suppl 258 1976
- 26 Touwen B C & Precht F R The neurological examination of the child with minor dysfunction *Clin Dev Med* 38 Spastics International Medical Publications 1970
- 27 Utbildningsdepartementet *Skolhälso- och sjukvårdens enkätundersökningar till berörda befattningsskylare* Ds U 1976 3 Gotab Stockholm 1976
- 28 Willner S *Factors contributing to structural scoliosis* Thesis Studentlitteratur Lund 1972
- 29 Yankauer A Allied health workers in pediatrics *Pediatrics* 41 1031 1968
- 30 Yankauer A Child health supervision is it worth it? *Pediatrics* 52 77 1973
- 31 Yankauer A & Lawrence R A A study of periodic school physical examinations *Am J Public Health* 51 1537 1961

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cure which they often do compared to nurses in many other countries. Their qualifications are comparable to university educated American nurse practitioners and health assistants who act as primary health care providers in preschool and school age in many parts of USA and have proved to be competent in health care as doctors or even better (7, 8, 11, 12, 22, 23, 29). The scoliosis screening in some school districts was delegated to nurses and resulted in a considerable increase of diagnosed cases of idiopathic scoliosis (1, 21).

The Swedish school nurses should be perfectly capable of handling some of the doctor's traditional work in school health.

From the present investigation it seems reasonable to suggest that physical screening of 10 to 12 year old school children should include

- 1 Assessment of the child's growth
- 2 Examination of the spine including the bending forward test for detection of scoliosis
- 3 Inspection of the skin

In connection with the examination information recorded concerning the child's health and well being should be collected from the children, their parents and teachers. Vision and hearing screening should also be made.

The school entrance health examination should probably still be undertaken by the doctor mainly because it would give him/her opportunity to establish a good contact with the child and its parents.

A rational economic use of medical resources is of course of utmost importance in health care planning. The methods used in this study reduce the costs for the school health without diminishing the efficiency. There is a considerable difference in salary between a doctor and a nurse US\$34/hour compared to US\$9/hour for the nurse (including employer's charge 44%, January 1979). The cost per child for a doctor's examination is approximately US\$2.8 and for a nurse's examination US\$0.75 (5 min/child or 12 children/hour). If the nurse examines the children first for

height and weight and visual acuity and assists the doctor when he makes his physical examination as is usual in Sweden the costs per 100 children will amount to US\$150 (nurse) + US\$280 (doctor) = US\$430. If the doctor examines only 20 children the costs will be US\$90 (nurse) + US\$56 (doctor) a reduction of costs by 60-70%.

Thus it is possible without requesting more resources to utilize the expensive school doctor for tasks for which his specialized skill is needed: medical care for acute and chronic illnesses, for health education to children, families and teachers and for a deeper concern about social and psychosocial aspects of the child's wellbeing which should also be covered by the health surveillance in school.

The school health service could obviously be a useful and highly estimated service. All children go to school, are easily accessible and through them also their homes and families. Thereby it is possible and also necessary to take a major responsibility for the children's health.

REFERENCES

- 1 Baker E. A. & Zingger B. School screening for idiopathic scoliosis. *Am J Nursing* 70: 767 1970.
- 2 Brundtland G. H. Skolen - sentralt angreppspunkt i forebyggende helsearbeid. In O. Larsen (ed.) *Forebyggende medisin*. Universitetsforlaget, Oslo 1975, p. 67.
- 3 *Child Health Report of a European symposium*. WHO, Copenhagen 1970.
- 4 Dunn B. H., Hakala M. W. & McGee M. E. Scoliosis screening. *Pediatrics* 61: 794 1978.
- 5 Fek K. & Iellenius G. *Skolhälsovård en handbok*. Liber Läromedel/Utbildningsförlaget, Stockholm 1976.
- 6 *Evaluation of School Health Programmes*. Report on a working group. WHO, Copenhagen 1978.
- 7 Fine L. L. The pediatric practice of the child health associate. *Am J Dis Child* 131: 634 1977.
- 8 Foye H., Chamberlin R. & Charney E. Content and emphasis of well child visits. Experienced nurse practitioners vs. pediatricians. *Am J Dis Child* 131: 794 1977.
- 9 Fog E. & Fog M. Cerebral inhibition examined by associated movements. *Clin Dev Med* (Heinemann, London) 10: 52 1963.
- 10 Frankenburg W. K. Pediatric screening. *Adv Pediatr* 20: 149 1973.

SELF CONTROL WITH URINALYSIS IN JUVENILE DIABETES

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ABSTRACT Ludvigsson J and Svensson P G (Department of Paediatrics University Hospital and Department of Sociology Linköping University Linköping Sweden) Self-control with urinalysis in juvenile diabetes. *Acta Paediatr Scand* 68 887 1979.—Urinary glucose excretion reflects the blood glucose levels and is therefore recommended and used as a relevant and practical method for self-control in juvenile diabetes. The purpose of this study was to estimate the attitudes of diabetic children and their parents towards such daily urinalysis. In 1975 69 juvenile diabetics 6-18 years old and their parents were studied and three years later another 69 patients were added. Standardized interviews and questionnaires were used. Only 3 out of 138 patients refused to test their urine regularly and to write down their results in the diary. The results indicate that a great majority of the patients and the parents easily accept the self testing method and regard it as a valuable tool in the management of the disease. Almost nobody experienced the urine tests as a psychological problem.

KEY WORDS Self control urinalysis juvenile diabetes attitudes

Diabetic children when in metabolic balance near normoglycemia feel subjectively well, grow normally, get normal pubertal development (14) and have a better defence mechanism against infections (1, 3). Most evidence supports the view that in such children late vascular complications are at least postponed (4, 7). Insulin, diet and physical exercise are the classical cornerstones to achieve such balance, but there is less agreement on the methods for evaluating the results of the treatment. Determinations of blood glucose levels or urinary glucose excretion in connection with sporadic visits to a diabetic clinic give only snapshots of reality. Haemoglobin A₁ reflects the longterm blood glucose levels better (5, 8) but only in retrospect and hence the information obtained through haemoglobin A₁ determinations comes too late and is of limited help in the regulation of the actual daily blood glucose variation. There is a need for a reliable and valid parameter giving day to day information about the patient's metabolic balance. For practical and psychological reasons one cannot recommend diabetic chil-

dren to have their blood glucose determined several times every day (2, 17). However, in patients with a normal renal threshold for glucose, urinary glucose excretion reflects the blood glucose levels (6, 10, 13, 15) and has therefore been recommended and used as a relevant and practical method for determination of the metabolic control (10, 11, 18). However, many physicians have been reluctant to accept this principle of daily urinalysis at home. Urinalysis is said to be unreliable, to in-

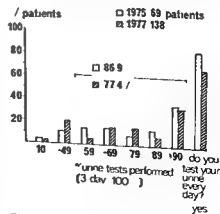


Fig 1 Frequency of urine tests performed

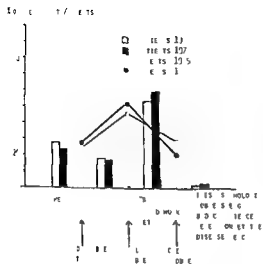


Fig 4 Answers to the question 'How do you/does he/she like the urine tests'

well as to age at onset ($p < 0.05$). A high frequency was positively correlated to the number of tests with no or minimal ($< 1\%$) glucosuria ($p < 0.01$) i.e. to better metabolic control (10).

Two years later 3 patients, all teenagers, out of 138 refused to test their urine regularly and to write down the results in the diary (Fig 1) although they declared that they did test their urine sporadically, especially when they did not feel well. There was no significant change in test frequencies between 1975 and 1977 although the proportion of patients who tested their urine $< 50\%$ of expected number had increased parallel to a decreasing proportion of patients who said they tested their urine every day (Fig 1). All patients who tested their urine $< 50\%$ of expected number were more than 12 years old both 1975 and 1977. Beside the drop in test frequency after the first two years there was no further decrease related to increasing duration (Fig 2).

In 1975 the attitudes towards the urinalysis were predominantly positive in 56 patients (83.6%). The patients' motivation for the treatment was positively correlated to the motivation of their parents ($p < 0.01$). A positive attitude towards the team at the diabetic

clinic was related to a good motivation for the treatment both among patients ($p < 0.01$) and parents ($p < 0.05$).

The attitude towards different parts of the treatment among the 121 patients interviewed in 1978 was compared to that of the 69 in 1975 in the answers to a question whether there was anything in the treatment that the patients did not like particularly (Fig 3). Although the proportion of patients who did not like the urinary tests had increased it was rather small and the parents' interpretation of their children's reaction is still more positive with regard to the urinary tests.

On the question 'How do you like the urine tests?' almost nobody experienced the urine tests as a heavy burden leading to bad conscience, psychological problems etc. However, about half of the patients think the tests are rather boring (Fig 4). The remaining patients either liked the tests well or accepted them. This pattern is in good agreement with the parents' estimate of the opinion of their children. Although many patients and parents thus think the urinary tests are boring, a great majority of both patients and parents feel that the urine tests are of value and useful (Fig 5). The practical usefulness of the urine testing was acknowledged by 74.6% of the parents in 1975 and by 94.8% in 1978. The proportion of parents who felt it important to test the child

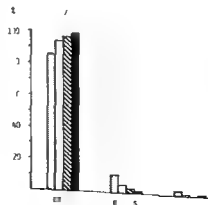


Fig 5 Answers to the question 'Do you think the urine tests are of value?' □ patients 1975, ■ patients 1978, □ parents 1975, ■ parents 1978

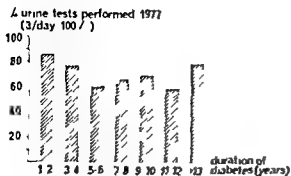


Fig. 2 Frequency of urine tests performed by the patients in relation to the duration of their disease

cause the anxiety of the patient and the fixation to the disease and to be unacceptable by the patients (12). The purpose of this study was for the first to determine the urine test frequency and correlate to some parameters and for the second to estimate the attitudes of diabetic children and their parents towards daily urinalysis at home and to correlate these attitudes to the actual urinalysis situation.

MATERIAL

Every diabetic child or adolescent in the hospital region is controlled at the diabetic clinic of the pediatric department. The patients are treated with insulin once or twice daily, regulated diet, and regular physical exercise. Since 1971 they have all been recommended to test single voided urine specimens at home with the Clinitest 2 drops method at least 3 times daily (before breakfast, before dinner in the afternoon, and before they go to bed) and in addition sometimes before lunch if practically possible. The urine is tested for ketone bodies by Ketostix when the patients have acute infections, are ill, do not feel well or have 2% glucose in the urine in repeated specimens. The results of the urine tests are registered in a special diary

which is analysed by the nurse and doctor at the hospital every 2-3 months.

In 1975 69 juvenile diabetics 6-18 years old were studied. Their age at onset of diabetes varied between 1-14 (6.4 ± 3.6) years and the duration of the disease between 3-17 (7.6 ± 3.3) years. Three years later another 6 patients were added. The age of the 134 patients varied between 1-21 years, age at onset of diabetes from 1-11 (7.3 ± 4.0) years and duration of diabetes from 1-17 (6.7 ± 3.6) years.

METHODS

In 1975 the attitudes towards the treatment were studied in two ways in all but two patients who were less than 7 years old. A standardized interview was constructed containing 52 questions related to the following items: Content of diet, regularity of diet, physical exercise, urinalysis at home, insulin injections, visits to the diabetic clinic, and regularity of daily activities. In addition a special attitude test was constructed which consisted of 34 provocative statements related to the same items (10). Each field was covered by negative and positive statements which the patients had to accept or reject whereby their attitudes could be classified from very negative to very positive. The special test was used as a control of the validity of the interviews. The result of the special attitude test used in 1975 was well correlated to the result of the interviews ($r=0.78$, $p<0.001$).

Three years later we used a questionnaire with the same questions as the standardized interviews. No answer was returned from 13 teenagers and 4 children were too young.

All diaries were collected and the frequency of performed urinary tests was counted.

RESULTS

Out of the 69 patients all but two teenagers tested their urine regularly during 1975 (Fig. 1).

1) The frequency of testing was negatively correlated to onset before 1970 ($p<0.05$) as

Fig. 3 Answer to the question 'Is there anything in the treatment you dislike especially much?'

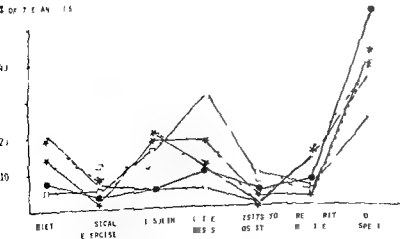


Fig. 3 Answer to the question 'Is there anything in the treatment you dislike especially much?' ★—★ patients 1975 ●—● patients 1978 ○—○ patients 1979 ★ ★ (=1975) and □ □ (=1978) shows what the parents believe their children dislike most

- controlled diabetic patients *Am J Med Sci* 763 451 1977
- 7 Bernstein R K Protein as the principal source of dietary carbohydrate for the tight control of glycemic levels in juvenile onset of diabetes Abstract 4th International Beilinson Symposium on Nutrition and the diabetic child Israel 1978
 - 8 Bybee J D & Rogers D E The phagocytic activity of polymorphonuclear leucocytes obtained from patients with diabetes mellitus *J Lab Clin Med* 61 1 1964
 - 9 Cahill G F Etzwiler D D & Freinkel N Control and diabetes *N Engl J Med* 294 1004 1976
 - 10 Ditzel J & Kjaegaard J J Haemoglobin A concentrations after initial treatment for newly discovered diabetes *B Med J* 1 741 1978
 - 11 Forman H H Goldstein H S & Genel M Management of juvenile diabetes mellitus usefulness of 4-hour fractional quantitative urine glucose *Pediatrics* 53 57 1974
 - 12 Jackson R L Juvenile diabetes In W J Malaisse & J Prart (eds) *Diabetes Excerpta Medica* Amsterdam 1974 p 50
 - 13 Koenig R J Peterson C M Jones H L Saudek C Lehrman M & Cerami A Correlation of glucose regulation and haemoglobin A_{1c} in diabetes mellitus *N Engl J Med* 795 417 1976
 - 14 Lanoe H Thibault N Eschwege E Soria J Soria C & Tchobrousky G Glycosylated haemoglobin concentrations and clinical results in insulin dependent diabetes *Lancet* ii 1156 1977
 - 15 Ludvigsson J Metabolic control in juvenile diabetes mellitus The influence of some clinical biochemical and socio-psychological factors Linköping University Medical Dissertations no 45 Zata tryckerierna Linköping 1976
 - 16 Ludvigsson J Hemmakontroll av urinen i behandlingen av juvenil diabetes mellitus In D Aagaard (ed) *Diabetes i barnearene* (Childhood diabetes) Oslo 1976
 - 17 Malone J I Hellrung J M Malphus F W Rosenbloom A L Gric A & Weber F T Good diabetic control—a study in mass delusion *J Pediatr* 89 943 1976
 - 18 Molnar G D Gastineau C F Rosevear J W & Morvess K E Quantitative aspects of labile diabetes *Diabetes* 14 779 1965
 - 19 Petersen H D Korsgaard B Deckert T & Nielsen E Growth body weight and insulin requirement in diabetic children *Acta Paediatr Scand* 67 453 1978
 - 20 Service F J Molnar G D & Taylor W F Urine glucose analyses during continuous blood glucose monitoring *JAMA* 277 294 1973
 - 21 Topper E Doron M Karp M & Laron Z The relationship between glycosylated haemoglobin and postprandial blood glucose in insulin independent juvenile diabetes Internat Diabetes Study Group Israel 1978
 - 22 Walford S Gale E A M Allison S P & Tattersall R H A pilot study of home blood glucose monitoring Abstract 4th International Beilinson Symposium on Nutrition and the diabetic child Israel 1978
 - 23 Weber B Validity and practicability of glucosuria self-control in juvenile diabetes In *The balance of diabetes in juveniles* International Beilinson Symposium Israel 1975
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dren's urine even in periods when the child feels well had also increased from 71% in 1975 to 95% in 1978. The growing feeling of usefulness of the diary for the patients and the parents themselves is also reflected by the answer to a question on the possibility of writing down a false or guessed figure instead of actually making the test. In 1975 19% thought this might happen but only 5% in 1978.

DISCUSSION

Several studies support the reliability and validity of daily determinations of glucose in single voided urine specimens through the Clinitest 2 drops method and this has been accepted as a relevant and useful method in the management of juvenile diabetes (6, 9, 10, 11, 16, 18). The three times recommended are chosen with regard to the school, but of course it is sometimes necessary to add a urine test before lunch to control the morning glucosuria.

This study shows that it is possible to get an overwhelming majority of patients to test their urine regularly for several years. A few teenagers refused totally while another group of teenagers tested their urine less frequently but still on an average almost once a day. The cooperation was quite as good as with regard to other parts of the treatment. The negative correlation between test frequency and onset before 1970 when urine tests were not recommended indicates that in order to get a good cooperation it is important to inform both patients and parents about the importance of the urine tests already at the onset of the disease. The negative correlation to higher age at onset reflects the increasing problems to get teenagers to cooperate. The correlation between high frequency and better metabolic control probably means both that urine tests contribute to better metabolic control and that it is more acceptable for those with a good metabolic control to test their urine.

The results obtained at the interviews in 1975 were validated through comparison with

a special attitude test. The reliability and validity of attitudes assessment tests are nevertheless uncertain and the results must be interpreted with caution. Furthermore in 1978 we got no answer from 13 teenagers whose attitudes reasonably were predominantly negative. Still with these facts in mind the results strongly indicate that without strict rules but with repeated information we may not have been able to make the urine testing pleasant. However although the patients disliked urine tests more than any other part of the treatment a great majority of the patients and the parents found the urine tests valuable and they accepted the urine tests as one of several necessary cornerstones in the treatment. Almost nobody experienced the urine tests as a psychological problem although it happens occasionally that we have to advise some patients to test less frequently when a too strong fixation to the disease seems to be developing. Our main impression is that daily home testing of the urine predominantly means psychological advantages. The patients or parents get the possibility to manage the treatment, they learn the effects of diet, physical exercise, insulin, infections etc. and can avoid acute complications. Thus the urine tests contribute to increasing knowledge, increasing confidence and independence, a more positive attitude to other parts of the treatment and as a consequence a better metabolic control. The facts that urine tests during periods are rejected by some teenagers and badly informed parents or may be taken too seriously by a few others are no justifications for rejecting this important part of the treatment of juvenile diabetes.

ACKNOWLEDGEMENTS

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REFERENCES

- 1 Bagdade J, Nielson K L & Bulech R J. Reversible abnormalities in phagocytic function in poorly

DIAGNOSIS OF HIRSCHSPRUNG'S DISEASE

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ABSTRACT Kekomäki M, Rapola J and Louhiimo I (The Children's Hospital, Helsinki University Central Hospital, Helsinki, Finland). Diagnosis of Hirschsprung's disease. *Acta Paediatr Scand* 68: 893-897, 1979.—A prospective study of the accuracy of various diagnostic methods used in the detection of Hirschsprung's disease (syn. congenital intestinal aganglionosis, CIA) in 80 consecutive infants and children was done during the period 1972-76. Every patient underwent a barium enema, a rectal mucosal biopsy, which was prepared for both the demonstration of ganglia and for the assessment of acetylcholinesterase activity (ACE), and anal manometry was performed. In evaluating the clinical history, special emphasis was placed on signs of neonatal ileus. In the group of 10 patients with a definite diagnosis of CIA, the results were almost uniform. In the non-CIA group, the search for ganglia in biopsy material proved non-confirmatory in nearly half of the cases studied due to the fact that specimens were taken too superficially. The findings pertaining to ACE, barium enema and the results of manometry were at variance or inconclusive of a final diagnosis in 10, 16 and 22% of the performed studies, respectively. The value given to neonatal history proved to be of the same order, i.e. 0% proved to be falsely positive.

KEY WORDS Megacolon, intestinal occlusion, manometry, acetylcholinesterase.

Since the recognition of the absence of ganglion cells in the intrinsic nerve plexuses of the rectum is a *conditio sine qua non* of Hirschsprung's disease (syn. congenital intestinal aganglionosis, CIA), a search for ganglion cells in the myenteric and submucosal nerve plexuses of the terminal rectum has served as a method of diagnosis in CIA. Two factors limit the usefulness of rectal biopsy. The taking of a too superficial biopsy does not always yield an adequate sample for the establishment of the presence of ganglion cells, and a full thickness rectal biopsy carries the risk of complications. An X-ray examination of the rectum with a barium enema also has its limitations, especially in short segment disease and also in higher lesions at an early age, when megacolon has not yet developed (5, 18). Therefore, other indirect methods have been introduced in order to arrive at a tentative diagnosis of CIA. These include the determination of the reflex functions of the internal sphincter of the rectum by anal tonometry (9, 14) and a histochemical investigation of the distribution of

acetylcholinesterase (ACE) in the muscularis mucosae and lamina propria of the rectal mucosa (11). We present here the results of investigations of 60 cases in which these techniques have been employed in the diagnosis of CIA.

PATIENTS AND METHODS

Patients. The material consisted of 60 consecutive patients with severe constipation examined at the Children's Hospital, Helsinki University Central Hospital during the years 1972-76. All these patients were subjected to (1) barium enema, (2) anal manometry and (3) suction or punch biopsy of the rectal mucosa. The sex of the patients, their age at the time of the investigation and the final clinical diagnosis, whether CIA or non-CIA, are given in Fig. 1. In operated cases, the final diagnosis of CIA was established by a histologic examination of the resected bowel. Symptoms and signs suggestive of intestinal obstruction (abdominal swelling, marked constipation and/or vomiting, meconium ileus and spontaneous perforation of the gut) were regarded as neonatal symptoms and signs suggestive of CIA (18).

Methods. A barium enema without any preceding evacuation of the rectum was performed (17). Tension changes in the internal sphincter were recorded by means of a simple self-constructed anal tonometer (4). A decrease in the sphincteric tone, ranging from 5 to 15 mmHg

Table 1 Neonatal history and results of investigations of 10 patients treated operatively for CIA

Positivity (+) signifies findings suggestive of CIA

Patient		Age (years)	Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
No	Sex							
1	♂	7/57	Ileus	+	+	(*)	+	-
	♂	7/57	Ileus	+	()	+	+	-
3	♂	1/17	Ileus sepsis?	+	+	+	+	21 transomy
4	♂	4/17	Vomiting diarrhea	+	+	(*)	+	-
5	♀	9/1	Sepsis? Mal absorption?	+	+	+	+	-
6	♂	1	Sepsis	+	+	+	+	-
7	♂	1	Vomiting	+	+	(*)	+	-
8	♂	1	Ileus	+	-	+	+	-
9	♂	7	Vomiting sepsis	+	+	+	+	-
10	♀	11	Vomiting	+	+	+	+	-

Technical difficulties in manometry

* Amount of submucosa judged insufficient to allow a definite diagnosis

ures in the manometry 29 patients out of 50 had a completely normal pattern of findings combined with a normal neonatal history. Of the remaining 21, 7 had defecation difficulties during the immediate neonatal period as the only feature suggestive of CIA. This group included 2 patients with a low (translevator) and rectal malformation, 2 patients with idiopathic perforation of the colon, 2 patients with minor defecation difficulties and one patient with a meconium plug syndrome.

Eight patients in the non CIA group had one pathological finding as regards barium enema, manometry or abnormal histology of the biopsy material (Table 2).

In 6 of the non CIA patients two or three diagnostic features out of the five were considered suggestive of CIA. Their detailed distribution is given in Table 3.

Table 2 Sporadic findings suggestive of CIA in 50 non CIA infants and children

Technique of examination	Number of patients
Barium enema	3
Manometry	3
Search for ganglia	1
ACE	1
Total	8

DISCUSSION

Classically the diagnosis of CIA is based on a typical clinical history, a barium enema and a full thickness rectal biopsy. Manometry and the determination of the distribution of ACE in the rectal mucosa have been recommended in recent years as a screening procedure for patients prior to more extensive examinations. Whatever the examination technique employed, the value of the results obtained appears to be limited by the age and condition of the patient (7, 13, 16). However, a more serious limitation is that all the techniques are prone to both technical failures and errors of interpretation.

The main aim of our study was to examine the possibility that the diagnostic accuracy of CIA could be improved by the performance of mutually independent methods of investigation. Referring to Table 4 we are able to answer this question in the affirmative.

Of the 10 patients with CIA, at least four abnormal findings out of five were considered to indicate CIA. In fact, discrepant findings were limited to only one case with a normal manometric tracing (Table 1).

The non CIA patients can be divided into two subgroups. The first consists of patients (44/50) with none or only one finding sug-

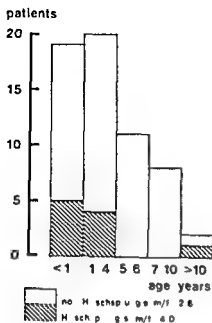


Fig. 1 Age distribution and sex ratio of patients

was interpreted as a normal response. Lack of internal sphincter inhibition or a slight increase in pressure were interpreted as abnormal. Resting pressure was not routinely recorded. To establish the reliability of the results manometry was repeated 4-5 times during the same hypnosis (Ketalar® Pirke Davis & Co. Pontypool Mon. U.K. 4-5 mg/kg b.wt.).

At least two specimens containing sufficient submucosa were taken at the level of anal valves (pectinate line). One of the samples was spread on a piece of absorbent paper with the mucosal surface upwards and fixed in formalin. Serial sections were used to search for submucosal ganglion cells. Another specimen was frozen rapidly in liquid nitrogen. Sections were stained for the histochemical determination of ACE (11). The presence of ganglia was determined in those paraffin sections which contained sufficiently submucosa. The sections stained for ACE were classified in three groups: (1) In normal mucosa there was faint and diffuse staining of the muscularis mucosae and a few thin positively staining nerve fibres at the border of the muscularis mucosae and submucosa. (2) The presence of ACE was considered positive (=suggestive of CIA) when there were many strongly positively staining nerve fibre bundles in the muscularis mucosae and numerous positively staining nerve fibres in the lamina propria (Fig. 2). (3) In a few cases an indeterminate pattern was found with occasional positively staining fibres in the lamina propria. This finding has been marked \pm in the tables.

RESULTS

Patients with CIA

The uniformity of the findings as regards the 10 patients with CIA was impressive (Table 1). In particular the barium enema results and the



Fig. 2 Distribution and activity of ACE pathognomonic for CIA. No counterstaining. Thick intensely stained nerve fibers are present in the submucosa (S) and muscularis mucosae (M). Thin positive fibres are seen in the lamina propria (L) $\times 200$.

determinations of ACE were uniformly in agreement with the final diagnosis. All histological specimens were found to be aganglionic. However, in one patient the result of the manometry was interpreted as being normal at repeated determinations.

Positive neonatal histories were found in all the patients studied with CIA. In none of the cases was this merely limited to mild constipation or abdominal swelling, but there were clear signs of intestinal obstruction necessitating hospitalization during the immediate neonatal period.

Patients with non CIA

Disregarding equivocal or negative findings due to obviously faulty technique such as biopsies too superficial for the search of ganglia to be made and purely instrumental fail

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No	Sex							
1	♂	1/5	Ileus	+	+	(*)	+	-
	♂	2/5 ²	Ileus	+	()	+	+	-
2	♂	1/1 ¹	Ileus sepsis ²	+	+	+	+	21 trisomy
4	♂	4/1	Vomiting diarrhea	+	+	(*)	+	-
5	♀	9/1	Sepsis ² Mal absorption ²	+	+	+	+	-
6	♂	1	Sepsis	+	+	+	+	-
7	♂	1	Vomiting	+	+	(*)	+	-
8	♂	1	Ileus	+	-	+	+	-
9	♂	2	Vomiting sepsis	+	+	+	+	-
10	♀	11	Vomiting	+	+	+	+	-

Technical difficulties in manometry

* Amount of submucosa judged insufficient to allow a definite diagnosis

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The main aim of our study was to examine the possibility that the diagnostic accuracy of CIA could be improved by the performance of mutually independent methods of investigation Referring to Table 4 we are able to answer this question in the affirmative

Of the 10 patients with CIA at least four abnormal findings out of five were considered to indicate CIA In fact discrepant findings were limited to only one case with a normal manometric tracing (Table 1)

The non CIA patients can be divided into two subgroups The first consists of patients (44/50) with none or only one finding sug

Table 3 *Clustering of features suggestive of CIA in six patients from non CIA group*

Positivity (+) denotes findings suggestive of CIA

No	Patient		Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
	Sex	Age (years)						
1	♀	1/12	Normal	+	+	(^b)	+	Congenital hypothyroidism no symptoms
2	♂	2/12	Meconium	+	-	-	±	No symptoms
3	♀	3/12	Defecation difficulties	+	(+)	+	-	No symptoms
4	♀	6/12	Normal	+	-	-	±	21 trisomy
5	♂	1	Defecation difficulties	-	(^a)	(^b)	+	Uses laxatives irregularly
6	♂	8	Normal	+	-	-	±	Epidermolysis bullosa anal fissure

For footnotes see Table 1

gestive of CIA. In the light of our experience these patients may be treated conservatively.

The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA constitute a diagnostic dilemma (Table 3). The diversity of the findings may reflect the inclusion of a few cases with an ultrashort ganglionic segment. It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings: if conservative treatment fails, incorrect myectomy is

performed both for diagnostic and therapeutic purposes (10-12).

The percentage of biopsies taken too superficially for proper evaluation is much higher than that reported by Campbell & Noblett (3) for example. This may reflect our wish not to perform too deep biopsies, especially in an asymptomatic neonate. Despite our carefulness we saw one serious complication in this series: a rectal perforation in a neonate occurring during suction biopsy. Significant and even lethal bleeding can complicate a punch biopsy (13-15) though more rarely than in the case of open surgical biopsy.

In our experience the results obtained by manometry appeared to be compatible with those reported by Aaronson & Nixon (1). However, to limit the technical pitfalls in manometry it seems to be essential to have balloon systems of different sizes. A one size proposal is also suggested by El-Shriefi et al. (4).

The determination of acetylcholinesterase in the rectal mucosa is probably the most significant advancement in the diagnosis of CIA. Meier Ruge et al. (11) are credited for the introduction of ACE determinations in clinical practice. Also in this series ACE determinations proved to be a method of great value. However, its specificity was somewhat reduced by the finding of an intermediate form of ACE distribution in three cases (Table 3).

Table 4 *Distribution of symptoms and clinical findings in 60 infants and children with suspected CIA*

The features evaluated are:

- signs of neonatal occlusion
- absence of ganglia in rectal wall
- ACE in rectal lumen proper
- long interval in barium enema and
- lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
5	5	CIA
4	5	CIA
3	3	non-CIA
2	3	non-CIA
1	15	non-CIA
0	29	non-CIA

Otherwise our results are in agreement with the original observations of Meier Ruge et al. Series with discordant results have also been published (6-19). However, in a recent appraisal of a large series the determination of ACE was found to be more reliable and consistent than any of the other methods available for the diagnosis of CIA (8).

Consequently the two latest non-invasive techniques employed in the diagnosis of CIA, manometry and ACE, seem to be of equal value to the classic ones. They are recommended for routine use at least in borderline cases.

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REFERENCES

- Aaronson I & Nixon H H. A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung's disease. *Gut* 13: 138, 1972.
- Aldridge R I & Campbell P E. Ganglion cell distribution in the normal rectum and anal canal: A basis for the diagnosis of Hirschsprung's disease by anorectal biopsy. *J Pediatr Surg* 3: 475, 1968.
- Campbell P E & Noble H R. Experience with rectal suction biopsy in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 4: 410, 1969.
- El Shafie M, Suzuki H, Schnauffer L, Haller J A & White J J. A simplified method of anorectal manometry for wider clinical application. *J Pediatr Surg* 7: 30, 1972.
- Ehrenpreis T. Megacolon in the newborn: A clinical and roentgenological study with special regard to the pathogenesis. *Acta Chir Scand* 94 (Suppl 227): 1946.
- Garrett J R, Howard E R & Nixon H H. Histochemical diagnosis of Hirschsprung's disease. *Lancet* II: 436, 1969.
- Holschneider A M, Kellner E, Streibl P & Sippell W G. The development of anorectal continence and its significance in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 11: 151, 1976.
- Lake B D, Pun P, Nixon H H & Claireaux A E. Hirschsprung's disease: An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis. *Arch Pathol Lab Med* 107: 744, 1978.
- Lawson J O N & Nixon H H. Anal canal pressures in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 2: 544, 1967.
- Lynn H H. Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon. *Z Kinderchir* 5 (Suppl): 98, 1968.
- Meier Ruge W, Lutterbeck P M, Herzog B, Morger R, Moser H & Scharli A. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7: 11, 1972.
- Nissan S, Bar Maor J A & Levy E. Anorectal myectomy in the treatment of short segment Hirschsprung's disease. *Ann Surg* 170: 969, 1969.
- Pease P W, Corcory J J & Cameron A H. Diagnosis of Hirschsprung's disease by punch biopsy of rectum. *Arch Dis Child* 51: 541, 1976.
- Schnauffer L, Talbert J L, Haller J A, Reid N C R W, Tabon F & Schuster M M. Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood. *J Pediatr Surg* 2: 538, 1967.
- Shandling B & Auldust A W. Punch biopsy of the rectum for the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7: 386, 1968.
- Smith H. Pre and postnatal development of the ganglion cells of the rectum and its surgical implications. *J Pediatr Surg* 3: 386, 1968.
- Swenson O, Neuhauser B D & Pickett L H. New concepts of etiology, diagnosis and treatment of congenital megacolon. *Pediatrics* 4: 401, 1949.
- Swenson C, Sherman J O & Fisher J H. Diagnosis of congenital megacolon: an analysis of 501 patients. *J Pediatr Surg* 8: 587, 1973.
- Trigg P H, Belin R, Haberkorn S, Long W J, Nixon H H, Plachkes J, Spitz L & Willital G H. Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung's disease. *J Clin Pathol* 27: 407, 1974.

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Table 3 *Clustering of features suggestive of CIA in six patients from non CIA group*

Positivity (+) denotes findings suggestive of CIA

No	Patient		Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
	Sex	Age (years)						
1	♀	1/12	Normal	+	+	(*)	+	Congenital hypothyroidism no symptoms
2	♂	2/12	Metrorrhoea	+	-	-	±	No symptoms
3	♀	3/12	Defecation difficulties	+	()	+	-	No symptoms
4	♀	6/12	Normal	+	-	-	±	21 trisomy
5	♂	1	Defecation difficulties	-	()	(*)	+	Uses laxatives irregularly
6	♂	8	Normal	+	-	-	±	Epidermolysis bullosa anal fissure

For footnotes see Table 1

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The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA constitute a diagnostic dilemma (Table 3). The diversity of the findings may reflect the inclusion of a few cases with an ultrashort aganglionic segment. It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings: if conservative treatment fails, anorectal myectomy is

performed both for diagnostic and therapeutic purposes (10-12).

The percentage of biopsies taken too superficially for proper evaluation is much higher than that reported by Campbell & Noblett (3). For example, this may reflect our wish not to perform too deep biopsies especially in an asymptomatic neonate. Despite our carefulness we saw one serious complication in this series: a rectal perforation in a neonate occurring during suction biopsy. Significant and even lethal bleeding can complicate a punch biopsy (13-15) though more rarely than in the case of open surgical biopsy.

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Table 4 *Distribution of symptoms and clinical findings in 26 infants and children with suspected CIA*

The features evaluated are

- signs of neonatal occlusion
- presence of ganglia in rectal wall
- ACE in rectal lamina propria
- long anal canal in barium enema and
- lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
5	5	CIA
4	5	CIA
3	3	non CIA
2	3	non CIA
1	15	non-CIA
0	29	non-CIA

Otherwise our results are in agreement with the original observations of Meier Ruge et al. Series with discordant results have also been published (6-19). However, in a recent appraisal of a large series the determination of ACE was found to be more reliable and consistent than any of the other methods available for the diagnosis of CIA (8).

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REFERENCES

- 1 Aaronson I & Nixon H H. A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung's disease. *Gut* 13 138 1972.
- 2 Aldridge R I & Campbell P E. Ganglion cell distribution in the normal rectum and anal canal: A basis for the diagnosis of Hirschsprung's disease by anorectal biopsy. *J Pediatr Surg* 3 475 1968.
- 3 Campbell P E & Noble H R. Experience with rectal suction biopsy in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 4 410 1969.
- 4 El Shafie M, Suzuki H, Schnauffer L, Haller J A & White J J. A simplified method of anorectal manometry for wider clinical application. *J Pediatr Surg* 7 30 1972.
- 5 Ehrenpreis T. Megacolon in the newborn: A clinical and roentgenological study with special regard to the pathogenesis. *Acta Chir Scand* 94 Suppl 112 1946.
- 6 Garrett J R, Howard E R & Nixon H H. Histochemical diagnosis of Hirschsprung's disease. *Lancet* II 436 1969.
- 7 Holschneider A M, Kellner E, Streibl P & Sippell W G. The development of anorectal continence and its significance in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 11 151 1976.

- 8 Lake B D, Puri P, Nixon H H & Claireaux A E. Hirschsprung's disease: An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis. *Arch Pathol Lab Med* 102 743 1978.
- 9 Lawson J M N & Nixon H H. Anal canal pressures in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 2 544 1967.
- 10 Lynn H B. Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon. *Z Kinderchir* 5 Suppl 98 1968.
- 11 Meier Ruge W, Lutterbeck P M, Herzog B, Morger R, Moser R & Scharh A. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7 11 1972.
- 12 Nissan S, Bar Maor J A & Levy E. Anorectal myomectomy in the treatment of short segment Hirschsprung's disease. *Ann Surg* 170 969 1969.
- 13 Pease P W, Corcoran J J & Cameron A H. Diagnosis of Hirschsprung's disease by punch biopsy of rectum. *Arch Dis Child* 51 541 1976.
- 14 Schnauffer L, Talbert J L, Haller J A, Reid N C R W, Tabon F & Schuster M M. Differential sphincteric studies in the diagnosis of anorectal disorders of childhood. *J Pediatr Surg* 2 538 1967.
- 15 Shandling B & Auldurst A W. Punch biopsy of the rectum for the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7 386 1968.
- 16 Smith B. Pre and postnatal development of the ganglion cells of the rectum and its surgical implications. *J Pediatr Surg* 3 386 1968.
- 17 Swenson O, Neuhauser M B D & Pickett L K. New concepts of etiology, diagnosis and treatment of congenital megacolon. *Pediatr* 103 4 701 1949.
- 18 Swenson C, Sherman J O & Fisher J H. Diagnosis of congenital megacolon: an analysis of 501 patients. *J Pediatr Surg* 8 587 1973.
- 19 Trigg P H, Belin R, Haberkorn S, Long W J, Nixon H H, Plaschkes J, Spitz L & Willital G H. Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung's disease. *J Clin Pathol* 27 67 1974.

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Table 3 Clusterings of features suggestive of CIA in six patients from non CIA group

Positivity (+) denotes findings suggestive of CIA

No	Patient		Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
	Sex	Age (years)						
1	♀	1/12	Normal	+	+	(^b)	+	Congenital hypothyroidism no symptoms
2	♂	2/12	Meteorismus	+	-	-	±	No symptoms
3	♀	3/12	Defecation difficulties	+	()	+	-	No symptoms
4	♀	6/12	Normal	+	-	-	±	21 trisomy
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In our experience the results obtained by manometry appeared to be compatible with those reported by Aaronson & Nixon (1). However, to limit the technical pitfalls in manometry, it seems to be essential to have balloon systems of different sizes at one's disposal, as also suggested by El Shafie et al (4).

The determination of acetylcholinesterase in the rectal mucosa is probably the most significant advancement in the diagnosis of CIA. Meier Ruge et al (11) are credited for the introduction of ACE determinations in clinical practice. Also in this series ACE determinations proved to be a method of great value. However, its specificity was somewhat reduced by the finding of an intermediate form of ACE distribution in three cases (Table 3).

Table 4 Distribution of symptoms and clinical findings in 60 infants and children with suspected CIA

The features evaluated are

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- lack of reflex relaxation of the internal sphincter in manometry

Number of positive symptoms and findings	Number of patients	Final clinical diagnosis
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Otherwise our results are in agreement with the original observations of Meier Ruge et al. Series with discordant results have also been published (6-19). However in a recent appraisal of a large series the determination of ACE was found to be more reliable and consistent than any of the other methods available for the diagnosis of CIA (8).

Consequently the two latest non-invasive techniques employed in the diagnosis of CIA, manometry and ACE, seem to be of equal value to the classic ones. They are recommended for routine use at least in borderline cases.

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REFERENCES

- 1 Aaronson I & Nixon H H. A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung's disease. *Gut* 13 138 1972.
- 2 Aldridge R I & Campbell P E. Ganglion cell distribution in the normal rectum and anal canal: A basis for the diagnosis of Hirschsprung's disease by anorectal biopsy. *J Pediatr Surg* 3 475 1968.
- 3 Campbell P E & Noblett H W. Experience with rectal suction biopsy in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 4 410 1969.
- 4 El Shafie M, Suzuki H, Schnauffer L, Haller J A & White J J. A simplified method of anorectal manometry for wider clinical application. *J Pediatr Surg* 7 730 1972.
- 5 Ehrenpreis T. Megacolon in the newborn: A clinical and roentgenological study with special regard to the pathogenesis. *Acta Chir Scand* 94 Suppl 117 1946.
- 6 Garrett J R, Howard E R & Nixon H H. Histochemical diagnosis of Hirschsprung's disease. *Lancet* II 436 1969.
- 7 Holschneider A M, Kellner M, Streibl P & Sippell W G. The development of anorectal continence and its significance in the diagnosis of Hirschsprung's disease. *J Pediatr* 5 11 151 1976.

- 8 Lake B D, Pan P, Nixon H H & Claireaux A E. Hirschsprung's disease: An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis. *Arch Pathol Lab Med* 107 244 1978.
- 9 Lawson J O N & Nixon H H. Anal canal pressures in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 2 544 1967.
- 10 Lynn H H. Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon. *Z Kinderchir* 5 Suppl 98 1968.
- 11 Meier Ruge W, Lutterbeck F M, Herzog B, Morger R, Moser R & Scharli A. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7 11 1972.
- 12 Nissan S, Bar Maor J A & Levy E. Anorectal myomectomy in the treatment of short segment Hirschsprung's disease. *Ann Surg* 170 969 1969.
- 13 Pease P W, Corcory J J & Cameron A H. Diagnosis of Hirschsprung's disease by punch biopsy of rectum. *Arch Dis Child* 51 541 1976.
- 14 Schnauffer L, Talbert J L, Haller J A, Reid M C R W, Tabon F C, Schuster M M. Differential sphincteric studies in the diagnosis of ano-rectal disorders of childhood. *J Pediatr Surg* 2 538 1967.
- 15 Shandling B & Auldust A W. Punch biopsy of the rectum for the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7 386 1968.
- 16 Smith B. Pre- and postnatal development of the ganglion cells of the rectum and its surgical implications. *J Pediatr Surg* 3 186 1968.
- 17 Swenson O, Neuhauser E H D & Pickett L K. New concepts of etiology, diagnosis and treatment of congenital megacolon. *Pediatrics* 4 701 1949.
- 18 Swenson C, Sherman J O & Fisher J H. Diagnosis of congenital megacolon: an analysis of 501 patients. *J Pediatr Surg* 8 587 1973.
- 19 Tngg P H, Behn R, Haberkorn S, Long W J, Nixon H H, Paschkes J, Spitz L & Willital G H. Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung's disease. *J Clin Pathol* 27 07 1974.

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Table 3 Clustering of features suggestive of CIA in six patients from non CIA group

Positivity (+) denotes findings suggestive of CIA

No	Patient		Neonatal history	Barium enema	Manometry	Search for ganglia	ACE	Note
	Sex	Age (years)						
1	♀	1/12	Normal	+	+	(*)	+	Congenital hypothyroidism no symptoms
2	♂	2/12	Meteorismus	+	-	-	±	No symptoms
3	♀	3/12	Defecation difficulties	+	()	+	-	No symptoms
4	♀	6/12	Normal	+	-	-	±	21 trisomy
5	♂	1	Defecation difficulties	-	()	(*)	+	Ureteric fistulae irregularly
6	♂	II	Normal	+	-	-	±	Epidermolysis bullosa anal fissure

For footnotes see Table 1

gestive of CIA. In the light of our experience these patients may be treated conservatively.

The second subgroup containing 6 infants and children with two or three diagnostic features indicating CIA constitute a diagnostic dilemma (Table 3). The diversity of the findings may reflect the inclusion of a few cases with an ultrashort aganglionic segment. It is therefore our present practice to stage therapy according to the clinical picture in patients with discrepant findings: if conservative treatment fails, anorectal myectomy is

performed both for diagnostic and therapeutic purposes (10, 12).

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REFERENCES

- 1 Aaronson I & Nixon H H. A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung's disease. *Gut* 13: 138, 1972.
- 2 Aldridge R I & Campbell P E. Ganglion cell distribution in the normal rectum and anal canal: A basis for the diagnosis of Hirschsprung's disease by anorectal biopsy. *J Pediatr Surg* 3: 475, 1968.
- 3 Campbell P E & Noble H R. Experience with rectal suction biopsy in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 4: 410, 1969.
- 4 El Shafie M, Suzuki H, Schnauffer L, Haller J A & White J J. A simplified method of anorectal manometry for wider clinical application. *J Pediatr Surg* 7: 730, 1972.
- 5 Ehrenpreis T. Megacolon in the newborn: A clinical and roentgenological study with special regard to the pathogenesis. *Acta Chir Scand* 94: Suppl 112, 1946.
- 6 Garrett J M, Howard E R & Nixon H H. Histochemical diagnosis of Hirschsprung's disease. *Lancet* II: 436, 1969.
- 7 Holschneider A M, Kellner E, Streibl P & Spill W G. The development of anorectal continence and its significance in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 11: 151, 1976.
- 8 Lake H D, Puri P, Nixon H H & Claireaux A E. Hirschsprung's disease: An appraisal of histochemically demonstrated acetylcholinesterase activity in suction rectal biopsy specimens as an aid to diagnosis. *Arch Pathol Lab Med* 107: 244, 1978.
- 9 Lawson J O N & Nixon H H. Anal canal pressures in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 2: 544, 1967.
- 10 Lynn H H. Personal experience with rectal myectomy in the treatment of selected cases of aganglionic megacolon. *Z Kinderchir* 5: Suppl 98, 1968.
- 11 Meier Ruge W, Lutterbeck P M, Herzog H, Morger H, Moser R & Scharli A. Acetylcholinesterase activity in suction biopsies of the rectum in the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7: 11, 1972.
- 12 Nissan S, Bar-Maor J A & Levy E. Anorectal myomectomy in the treatment of short segment Hirschsprung's disease. *Ann Surg* 170: 969, 1969.
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- 14 Schnauffer L, Talbert J L, Haller J A, Reid N C H, W. Tabon F & Schuster M M. Differential sphincteric studies in the diagnosis of anorectal disorders of childhood. *J Pediatr Surg* 2: 538, 1967.
- 15 Shandling H & Auldust A W. Punch biopsy of the rectum for the diagnosis of Hirschsprung's disease. *J Pediatr Surg* 7: 386, 1968.
- 16 Smith B. Pre and postnatal development of the ganglion cells of the rectum and its surgical implications. *J Pediatr Surg* 3: 386, 1968.
- 17 Swenson O, Neuhauser E B H & Pickett L K. New concepts of etiology, diagnosis and treatment of congenital megacolon. *Pediatrics* 4: 01, 1949.
- 18 Swenson C, Sherman J O & Fisher J H. Diagnosis of congenital megacolon: an analysis of 501 patients. *J Pediatr Surg* 8: 587, 1973.
- 19 Trigg P H, Belin R, Haberkorn S, Long W J, Nixon H H, Plaschkes J, Spitz L & Willital G H. Experience with a cholinesterase histochemical technique for rectal suction biopsies in the diagnosis of Hirschsprung's disease. *J Clin Pathol* 27: 707, 1974.

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EFFECT OF PREGNANCY ANAEMIA ON CELLULAR GROWTH IN THE HUMAN PLACENTA

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*From the Paediatric Haematology Unit Department of Paediatrics
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ABSTRACT Marwah P Singla P N Krishna M and Agarwal K N (Paediatric Haematology Unit Department of Paediatrics Institute of Medical Sciences Varanasi India) Effect of pregnancy anaemia on cellular growth in the human placenta *Acta Paediatr Scand* 68 899 1979—The effects of pregnancy anaemia on cell number and size in human placenta were investigated. Total organ weight, protein, RNA and DNA contents were determined in 54 fresh placentae from anaemic women (haemoglobin <110 g/l) and another 32 placentae from women without anaemia (haemoglobin ≥ 110 g/l). The placental weight was significantly reduced in pregnancy anaemia. The decrease in total placental DNA in anaemic women suggested that the reduced placental weight was due to a decrease in cell number. However, these placentae also showed evidence of compensatory cellular hypertrophy as indicated by increase in both weight per cell and protein per cell.

KEY WORDS Pregnancy anaemia, placenta, RNA, DNA.

Several investigators have attempted to elucidate biochemical and physiologic mechanisms by which the placenta is able to support the growth and development of the foetus in maternal anaemia (3, 8, 9, 11, 12). The placenta itself is a rapidly growing organ. However, only few studies (1, 2) have been carried out on placental growth in pregnancy anaemia and the actual cellular events occurring during this growth have not been elucidated. Since cellular DNA is located almost entirely within the nucleus and is constant in amount within the diploid nucleus of any species, the total quantity of DNA in a tissue or organ reflects the number of cells. The ratio of total organ weight or protein content to DNA content is a measure of cell size, and the RNA/DNA ratio determines the average RNA content per cell (4, 13, 14).

The objective of the present study was to determine the effects of pregnancy anaemia on cell division and cell growth in the human placenta utilizing the measurements of total organ weight, protein, RNA and DNA contents.

MATERIAL AND METHODS

Fifty-four fresh human placentae from anaemic women (haemoglobin <110 g/l) were studied. Another 32 placentae from women with haemoglobin 110 g/l or above at the time of delivery served as controls. All these women had singleton livebirths with gestation ranging from 37 to 41 weeks. They had no racial, cultural or environmental differences. The women with preterm delivery, antepartum haemorrhage, toxemia of pregnancy and blood group incompatibility were excluded from this study and so were women with systemic diseases which could have affected the placental growth.

The haemoglobin was estimated by cyanmethaemoglobin method (5) on venous blood taken during the first stage of labour. As soon as the placenta was delivered, the umbilical cord was cut flush with the placental surface and the membranes were trimmed off. Blood clots adhering to the placenta were removed and the subchorionic vessels were emptied of blood by gentle pressure. The placenta was blotted several times with filter paper and weighed.

For measurement of the placental protein, RNA and DNA contents, 1.0 g of the tissue was taken from the maternal surface of the placenta at a distance of about 1.0 cm from the cord and homogenized in 5.0 ml of ice-cold distilled water for 3 min using a Potter Elvehjem homogenizer fitted with a teflon pestle. Protein was determined in the homogenate by the method of Lowry et al. (7). RNA and DNA were determined using the method of Schneider (10).

Table 1 Placental weight and some biochemical characteristics in pregnancy anaemia (mean \pm S D)

Figures in parentheses indicate sample size

Group	Range of haemoglobin (g/l)	Placenta			
		Weight (g)	Protein (g)	RNA (mg)	DNA (mg)
I	≤ 60 (12)	337 \pm 73	52.3 \pm 14.6	946.6 \pm 352.8	1222.2 \pm 378.4
II	61-85 (15)	384 \pm 41	67.0 \pm 12.0	1275.9 \pm 323.0	1570.5 \pm 313.1
III	86-109 (27)	390 \pm 55	66.4 \pm 14.5	1310.3 \pm 415.3	1551.8 \pm 369.5
IV	≥ 110 (32)	430 \pm 46	74.3 \pm 20.6	1681.2 \pm 577.8	1946.8 \pm 478.7
Total observations (86)		397 \pm 61	67.5 \pm 17.9	1391.6 \pm 521.4	1656.1 \pm 472.7
p value					
I/IV		<0.001	<0.001	<0.001	<0.001
II/IV		<0.005	n.s.	<0.005	<0.005
III/IV		<0.005	n.s.	<0.01	<0.001

n.s. = not significant

RESULTS

Table 1 shows the distribution of women in four haemoglobin groups. These groups were comparable in terms of gestational age parity and absence of severe morbidity during pregnancy. It can be further seen that the placental weights and RNA and DNA values expressed per whole placenta were significantly low in women with various grades of anaemia (groups I, II & III) compared to the non anaemic women (group IV). The protein values expressed for whole placenta were however

significantly low only in the women with severe anaemia (haemoglobin ≤ 60 g/l).

Table 2 shows that the average placental weight/DNA and protein/DNA ratios were significantly high in the anaemic women while the average RNA/DNA ratio remained unaltered.

DISCUSSION

The results of the present study demonstrate that the placental weight is significantly low in anaemic women indicating that placental

Table 2 Effect of pregnancy anaemia on weight, protein and RNA per cell in the placenta (mean \pm S D)

Figures in parentheses indicate sample size

Group	Range of haemoglobin (g/l)	Placenta		
		Weight/DNA (g/g)	Protein/DNA (g/g)	RNA/DNA (mg/mg)
I	≤ 60 (12)	287.1 \pm 53.1	44.3 \pm 8.8	0.80 \pm 0.23
II	61-85 (15)	256.4 \pm 31.6	43.5 \pm 7.6	0.82 \pm 0.17
III	86-109 (27)	258.9 \pm 53.0	43.4 \pm 6.8	0.85 \pm 0.18
IV	≥ 110 (32)	231.2 \pm 48.7	38.1 \pm 6.3	0.85 \pm 0.17
Total observations (86)		252.1 \pm 51.4	41.6 \pm 7.8	0.84 \pm 0.18
p value				
I/IV		<0.005	<0.05	n.s.
II/IV		<0.05	<0.025	n.s.
III/IV		<0.05	<0.005	n.s.

n.s. = not significant

growth is retarded in pregnancy anaemia. This is contrary to the observations of Beischer et al. (7) and Agboola (1) that pregnancy anaemia is associated with a large placenta.

The lower DNA content in the placentae of anaemic women in this study indicates that the decreased organ size is a consequence of a decrease in cell number. Since cell division does not occur in the human placenta after 36 weeks of gestation (4, 6) it can be concluded that the decreased placental DNA content is due to earlier reduction in cell division. However, cell size as indicated by total organ weight/DNA and protein/DNA ratios is significantly increased in these placentae, suggesting cellular hypertrophy. The significance of placental cellular hypertrophy evoked by pregnancy anaemia is considerable because it may indicate an adaptation to a physiological stress resulting in an improvement of placental function and foetal well being.

The decrease in RNA content parallels the decrease in DNA content in the placentae of anaemic women. The resulting RNA/DNA ratio does not show any change indicating that the amount of RNA per cell in the placenta remains constant in pregnancy anaemia.

REFERENCES

- 1 Agboola A. Placental changes in patients with a low haematocrit. *Br J Obstet Gynaecol* 87: 25, 1975.
- 2 Beischer N A, Sivasubramanian R, Vohra S, Salpiromkosol M & Reid S. Placental hypertrophy in severe pregnancy anaemia. *J Obstet Gynaecol Br Comm* 77: 398, 1970.
- 3 Bothwell T H, Bothwell O, & Majumdar S & Finch C A. Iron metabolism in the pregnant rabbit: Iron transport across the placenta. *Am J Physiol* 193: 615, 1958.

- 4 Brasel J A & Winick M. Maternal nutrition and placental growth. Experimental studies of effects of maternal undernutrition on foetal and placental growth. *Arch Dis Child* 47: 479, 1972.
- 5 Crosby W H, Munn J I & Furth F W. Standardizing a method for clinical haemoglobinometry. *US Armed Forces Med J* 5: 693, 1954.
- 6 Laga E M, Driscoll S G & Munro H N. Comparison of placentas from two socio-economic groups. II. Biochemical characteristics. *Pediatrics* 50: 31, 1972.
- 7 Lowry O H, Rosebrough N J, Farr H I & Randall R J. Protein measurement with Folin phenol reagent. *J Biol Chem* 193: 265, 1951.
- 8 McLaurin L P & Cotter J R. Placental transfer of iron. *Am J Obstet Gynecol* 98: 931, 1967.
- 9 Nhonoh A M, Kihama F E & Ramu O O. The relation between maternal and cord serum iron levels and its effect on foetal growth in iron deficient mothers without malarial infection. *Br J Obstet Gynaecol* 87: 467, 1975.
- 10 Schneider W E. Determination of nucleic acids in tissues by pentose analysis. In S P Colowick & M O Kaplan (eds) *Methods in enzymology*. Academic Press Inc, New York, 1957, vol III, p 680.
- 11 Singla P N, Chand S, Khanna S & Agarwal K N. Effect of maternal anaemia on the placenta and the newborn infant. *Acta Paediatr Scand* 67: 645, 1978.
- 12 Singla P N, Chand S & Agarwal K N. Cord serum and placental tissue iron status in maternal hypochromia. *Am J Clin Nutr* (in press).
- 13 Winick M, Coscia A & Noble A. Cellular growth in human placenta. I. Normal placental growth. *Pediatrics* 39: 748, 1967.
- 14 Zeman F J & Stanbrough E C. Effect of maternal protein deficiency on cellular development in the fetal rat. *J Nutr* 99: 274, 1969.

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INCREASED OSMOTIC FRAGILITY OF ERYTHROCYTES IN CHRONICALLY JAUNDICED RATS AFTER PHOTOTHERAPY

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ABSTRACT Cukier J O Maglalang A C and Odell G B (Department of Pediatrics Johns Hopkins University School of Medicine Baltimore Maryland and the Department of Pediatrics University of Wisconsin School of Medicine Madison Wisconsin) Increased osmotic fragility of erythrocytes in chronically jaundiced rats after phototherapy *Acta Paediatr Scand* 68 903 1979.—Littermate homozygous (JJ) and heterozygous (Jj) Gunn rats were irradiated with blue fluorescent light for 18 hours continuously. The incident irradiance was 1.5 mWatts/cm in the 470-480 nm band pass. The influence of the irradiance on circulating erythrocytes was studied by testing their osmotic fragility before and after the irradiance. The non jaundiced Jj animals did not exhibit any increase in the osmotic fragility of their erythrocytes. The osmotic fragility of the erythrocytes from jaundiced JJ animals was the same as the Jj animals prior irradiance. However the fragility of the erythrocytes from the JJ animals was significantly increased after the 18 hours of irradiance. The results indicate that the photodynamic action of bilirubin may be present *in vivo*.

KEY WORDS Bilirubin phototherapy photosensitized hemolysis osmotic fragility Gunn rat

In a previous report it was suggested that phototherapy for neonatal hyperbilirubinemia might actually increase the formation of bilirubin because of the capacity of bilirubin to produce light induced hemolysis (22) *in vitro* and the failure of some infants treated by phototherapy to show significant reductions in their concentrations of serum bilirubin (26).

Since Saeki's original observations (29) it has been recognized that bilirubin is a photosensitizing agent and can produce hemolysis when within the erythrocyte in the presence of oxygen and light (22) i.e. it is a photodynamic agent (1). The basis for its photodynamic action has been verified by its ability to generate singlet oxygen (20) and thus resembles many photosensitizing hemolytic compounds (21-30). As with other photolytic compounds in order for bilirubin to induce photosensitized hemolysis it must be within the red-cell membrane (22-24).

Photosensitized hemolysis has been historically characterized into immediate hemolysis which is the hemolysis that occurs during the light exposure and delayed or after light hemolysis which reflects the continuing injury to the red cell even after the cessation of irradiance (1).

Associated with the hemolysis induced *in vitro* by bilirubin are alterations in the enzymatic activities of erythrocyte membranes. These include reduction in Mg^{2+} ATPase and Na^{+} K^{+} ATPase (9, 16, 22), acetylcholinesterase and glyceraldehyde 3 phosphate dehydrogenase (9, 10). Chemical alterations of photosensitized erythrocytes secondary to singlet oxygen (1O_2) formation have also been described with other porphyrin pigments and include peroxidation of unsaturated membrane lipids (12) and cholesterol (8) as well as polypeptide aggregation (11). Structural changes consist of the formation of spherocytes (22).

Table 1 Comparative responses of homozygous and heterozygous Gunn rats to 18 hrs of continuous phototherapy

Animal	Weight (g)	Hematoct (r)	Hemoglobin (g/dl)	Total plasma protein (g/dl)	Plasma bilirubin (mg/dl)
J ₁	239	50 ^a	15.3	5.5	4.7
J ₁	NW	47	16.0	5.8	3.0
J ₁	273	50	14.8	6.0	6.8
J ₁	243	49.5	14.0	6.0	6.8
J ₁	353	47	13.3	6.0	10.0
J ₁	345	50	14.7	6.0	7.5
J ₁	347	49.5	16.4	6.2	7.8
J ₁	337	46.5	15.2	6.0	7.5
J ₁	251	47.5	13.6	7.0	
J ₁	NW	44	12.4	7.0	
J ₁	563	51	13.7	6.5	
J ₁	535	51	16.2	6.5	
J ₁	400	50.5	16.2	6.0	
J ₁	379	51.5	16.0	6.0	
J ₁	382	49.5	16.2	6.0	
J ₁	373	48.0	15.7	6.2	

Not weighed ^a Pre phototherapy Post phototherapy

The translation of the above *in vitro* actions of bilirubin to *in vivo* circumstances must be done with caution for the action spectrum of bilirubin *in vivo* is not known and the wavelengths of light responsible for the reduction clinically in serum concentrations of bilirubin may not be the same as the wavelengths responsible for its photosensitizing hemolytic effects. The studies of Vogl (32) indicated that the radiant energy from broad spectrum fluorescent lights that are responsible for the reduction of skin bilirubin concentrations apparently penetrate no more than the first 2 mm of the skin. However *in vivo* for light to produce a photosensitized hemolysis the incident radiant energy must not only penetrate the epidermis but also extravascular tissues of the dermis and the vascular endothelium before it can reach circulating erythrocytes. Furthermore one might anticipate only those erythrocytes within the very periphery of the capillary lumen would be subject to the photosensitized hemolytic action of bilirubin for the hemoglobin within erythrocytes strongly absorb the radiant energy between 400–500 nm which are thought to be the wavelengths of light essential for the photodynamic effects

of bilirubin (31). Thus despite the well documented photolytic action of bilirubin on isolated erythrocytes *in vivo* circumstances may militate against any photodynamic hemolytic action in clinical circumstances.

In order to test the influence of the radiant energy of fluorescent lights used clinically for phototherapy on circulating erythrocytes *in vivo* we studied the integrity of erythrocytes from homozygous jaundiced (J₁) and littermate heterozygous (J₂) Gunn strain of rats subjected to 18 hours of continuous phototherapy with blue fluorescent lights. After completion of these studies contradictory reports have been published indicating that fluorescent irradiation of jaundiced Gunn rats does and does not influence red cell survival (14–28).

MATERIALS AND METHODS

Four pairs of non-icteric heterozygous (J₂) and jaundiced homozygous (J₁) littermates were selected and when the animals weighed 200 g they were splenectomized under ether anaesthesia. Four to six weeks after splenectomy the J₂ and J₁ littermates were again anaesthetized with ether and their dorsal and lateral skin removed by an electric clipper. While anaesthetized 0.8 ml of blood was collected from the tail into heparinized (2 ml capacity) glass tubes before and after the 18 hours of fluorescent

Table 2 The immediate and delayed osmotic fragility of erythrocytes from Gunn rats before and after 18 hours of continuous phototherapy

Osmolality (mOsm/kg)	Pre photo Rx		Post photo Rx	
	μ (n=4)	J_2 (n=4)	μ (n=4)	J_2 (n=4)
<i>Immediate hemolysis (%)</i>				
70	0.4 ± 0.17	0.7 ± 0.10	0.21 ± 0.11	0.16 ± 0.04
97	0.77 ± 0.16	0.1 ± 0.10	0.9 ± 0.09	0.30 ± 0.14
135	0.89 ± 0.8	0.55 ± 0.73	1.9 ± 0.81	0.45 ± 0.93
118	15.41 ± 9.50	1.19 ± 5.44	14.80 ± 10.83	13.73 ± 6.0
10	43.77 ± 8.91	57.90 ± 9.56	45.69 ± 13.19	4.67 ± 9.77
90	66.96 ± 13.7	74.3 ± 7.09	71.75 ± 19.07	71.71 ± 9.67
57	87.89 ± 13.7	87.65 ± 8.4	81.31 ± 13.76	86.17 ± 6.83
0	83.7 ± 14.61	93.01 ± 8.08	84.05 ± 1.30	86.08 ± 3.98
<i>Delayed hemolysis (%)</i>				
70	0.46 ± 0.77	0.48 ± 0.77	0.36 ± 0.18	0.37 ± 0.77
97	0.49 ± 0.18	0.57 ± 0.17	0.78 ± 0.69*	0.77 ± 0.08
135	1.77 ± 0.56	1.74 ± 0.40	7.98 ± 3.01	0.86 ± 0.15

* μ vs μ pre and post photo Rx $p < 0.05$

μ vs J_2 post photo Rx $p < 0.01$

μ vs J_2 post photo Rx $p < 0.001$

light irradiance. The tail of the anesthetized animal was warmed in hot tap water and the tip incised with a scalpel blade. The blood was collected by gentle milking of the incised tail permitting free flow into heparinized glass tubes. After the sample collection hemostasis was accomplished by coagulation by application of a pre heated metal spatula to the incised tail.

For the irradiance exposure the animals were placed in a plastic cage containing maple wood shavings and food pellets. The top of the cage was covered by a stainless steel slotted grid which supported a water bottle to which the animals had free access during the experiment. The fluorescent irradiance source was from a bank of 10 fluorescent lights (SE F'D T17/B); positioned 60 cm above the cage. The incident radiant energy at the top of the cage was 1.5 mWatts/cm between the 400-480 nm band pass. The light energy was measured with a bread board model selenium irradiance meter equipped with a neutral density and Wratten (no. 98) filters. Calibration of the light meter was done at the Applied Physics Laboratory of the Johns Hopkins University and the National Bureau of Standards (44). The duration of irradiance was 18 hours continuously.

The pre and post phototherapy blood samples were handled identically. The immediate and delayed light induced effects on the erythrocytes was quantitated by the changes in osmotic fragility after the method of Cartwright (4).

To 0.5 ml of phosphate buffered solutions of saline (pH 7.4) 50 μ l of whole blood was pipetted into duplicate sets of tubes. The osmolalities of the solutions ranged between 0 and 7 mOsm/kg. One set was allowed to stand immediately after mixing for 30 min at 35°C. The other set which consisted of blood samples added to buffered solutions of 0.07 and 135 mOsm/kg,

were covered and refrigerated at 4°C for 24 hours in the dark.

After 30 min of incubation the samples immediately studied were centrifuged at 3000xg for 10 min. From the supernatant appropriate aliquots were taken for determination of hemoglobin by the benzidine technique of Crosby (61). The loss of erythrocyte hemoglobin was calculated as percent of total hemoglobin from the supernatant of a blood sample that had been completely hemolyzed by distilled water. Whole blood rather than washed cells were used because in preliminary trials it was found that rat erythrocytes hemolyze while being washed in the absence of albumin.

The second set of samples which had been refrigerated 24 hours were brought to 25°C incubated 30 min and then analyzed as described above. The latter samples represented the delayed or after light injury to the erythrocyte population.

The osmolality of the phosphate buffered solutions of saline was measured in a vapor pressure osmometer (MicroLab model no. 301) at 37°C.

From the residual samples of heparinized blood the whole blood hematocrits were determined in duplicate by filling 70 μ l capillary hematocrit tubes and subsequently centrifuging them. The remaining blood was transferred to Natchon capillary tubes and centrifuged at 3000xg for 10 min. The supernatant plasmas were analyzed for total protein concentration by a biuret technique (13) and in the instances of the μ jaundiced animals, total bilirubin concentrations were measured colorimetrically after chemical conversion to the azodypyrrole sulfanilate derivatives (17).

The bilirubin content of the erythrocytes in jaundiced rats was measured in 5 animals of comparable age to the study animals. Under ether anesthesia blood was aspirated from the abdominal aorta into a heparinized

Table 1 *Comparative responses of homozygous and heterozygous Gunn rats to 18 hrs of continuous phototherapy*

Animal	Weight (g)	Hematocrit (%)	Hemoglobin (g/dl)	Total plasma protein (g/dl)	Plasma bilirubin (mg/dl)
Y ₁	239	50*	15.3	5.5	4.7
	NW*	47	16.0	5.8	3.0
Y	273	50	14.8	6.0	6.8
	243	49.5	14.0	6.0	6.8
Y ₁	353	47	13.3	6.0	10.0
	345	50	14.7	6.0	7.5
Y ₁	347	49.5	16.4	6.2	7.8
	337	46.5	15.2	6.0	7.5
J ₁	251	47.5	13.6	7.0	
	NW	44	12.4	7.0	
J ₂	463	51	13.7	6.5	
	535	51	16.2	6.5	
J ₂	400	50.5	16.2	6.0	
	379	51.5	16.0	6.0	
J ₁	342	49.5	16.2	6.0	
	373	48.0	15.7	6.2	

Not weighed * Pre phototherapy † Post phototherapy

The translation of the above *in vitro* actions of bilirubin to *in vivo* circumstances must be done with caution for the action spectrum of bilirubin *in vivo* is not known and the wavelengths of light responsible for the reduction clinically in serum concentrations of bilirubin may not be the same as the wavelengths responsible for its photosensitizing hemolytic effects. The studies of Vogl (32) indicated that the radiant energy from broad spectrum fluorescent lights that are responsible for the reduction of skin bilirubin concentrations apparently penetrate no more than the first 2 mm of the skin. However *in vivo* for light to produce a photosensitized hemolysis the incident radiant energy must not only penetrate the epidermis but also extravascular tissues of the dermis and the vascular endothelium before it can reach circulating erythrocytes. Furthermore, one might anticipate only those erythrocytes within the very periphery of the capillary lumen would be subject to the photosensitized hemolytic action of bilirubin for the hemoglobin within erythrocytes strongly absorb the radiant energy between 400–500 nm which are thought to be the wavelengths of light essential for the photodynamic effects

of bilirubin (31). Thus despite the well documented photolytic action of bilirubin on isolated erythrocytes *in vivo* circumstances may militate against any photodynamic hemolytic action in clinical circumstances.

In order to test the influence of the radiant energy of fluorescent lights used clinically for phototherapy on circulating erythrocytes *in vivo* we studied the integrity of erythrocytes from homozygous jaundiced (Y) and littermate heterozygous (J) Gunn strain of rats subjected to 18 hours of continuous phototherapy with blue fluorescent lights. After completion of these studies contradictory reports have been published indicating that fluorescent irradiation of jaundiced Gunn rats does and does not influence red cell survival (14–28).

MATERIALS AND METHODS

Four pairs of non-icteric heterozygous (J) and jaundiced homozygous (Y) littermates were selected and when the animals weighed 200 g they were splenectomized under ether anaesthesia. Four to six weeks after splenectomy the J and Y littermates were again anaesthetized with ether and their dorsal and lateral skin removed by an electric clipper. While anaesthetized 0.8 ml of blood was collected from the tail into heparinized (2 ml capacity) glass tubes before and after the 18 hours of fluorescent

membrane and a secondary colloid osmotic swelling of the erythrocyte due to its contained hemoglobin (5). The membrane pump(s) can no longer maintain the concentration gradient between intra and extra erythrocyte sodium. The oncotic effect of hemoglobin increases the erythrocyte volume and spherocytosis occurs which precedes the actual hemolysis.

The results in littermate Gunn rats demonstrated a difference in the osmotic fragility of erythrocytes from JJ and Jj animals after 18 hours of irradiance. The increased susceptibility to osmotic challenge of the erythrocytes from the JJ animals only indicate that the irradiance created a light induced injury unique to the jaundiced animals. Since the osmotic fragility was not increased when measured immediately, spherocytes were apparently not present *in vivo*. The spherocytosis occurred during *in vitro* storage of the light damaged cells.

Previous *in vitro* studies have demonstrated that if bilirubin is in the erythrocyte fluorescent irradiance can induce hemolysis in the presence of oxygen (22). In the current study it was possible to demonstrate that the circulating erythrocytes in JJ animals contained an average of 0.9 mg bilirubin/100 ml of packed cells. Confidence in the absolute value is subject to some reservation because its determination quantitatively depended on the small arithmetic difference between two larger values of plasma bilirubin concentrations. The differences would have greater significance if the cells could have been washed free of plasma before resuspension in albumin as done by Bralid (2) with human erythrocytes but unfortunately the rat erythrocytes hemolyzed in the absence of albumin. The large amount of hemoglobin present in the supernatant fluid of so washed erythrocytes rendered the determination of the small concentrations of bilirubin even less reliable.

Other investigations have also demonstrated alterations in circulating blood ele-

ments including thrombocytopenia (18) and reduction of erythrocyte ATPase (16) and acetyl salicylic acid esterase (33) associated with fluorescent irradiation *in vivo* of jaundiced infants. Such observations indicate that some of the incident irradiance associated with phototherapy may indeed penetrate into the capillary circulation.

The occurrence of increased production of bilirubin associated with phototherapy can be surmised from the earlier studies of Ostrow (25). The latter investigations using radiolabelled ^{14}C bilirubin in jaundiced rats demonstrated an 8–10 fold increase in biliary excretion of the radiolabel during fluorescent irradiance mostly as unconjugated bilirubin. These observations have been confirmed (19). An accelerated decay in the specific activity of the serum bilirubin (dpm/mg bilirubin) was also noted. However the serum bilirubin concentrations in some of the animals did not decrease. One probable explanation is that simultaneous to the increased excretion of bilirubin was an accelerated appearance of unlabelled bilirubin into the circulation. This latter unlabelled bilirubin was derived from accelerated heme protein catabolism. The present studies suggest one source of this unlabelled bilirubin was from the catabolism of hemoglobin from photosensitized erythrocytes. The increased reticulocytosis in jaundiced rats exposed to fluorescent irradiation (28) is also consistent with such an hypothesis. Thus the failure of animals JJ and JJ₁ in the present report to exhibit significant reductions in their serum bilirubin concentrations after 18 hours of irradiance may simply reflect an acceleration in the formation of bilirubin that equalled its excretion rather than a failure of phototherapy to induce accelerated hepatic excretion of bilirubin.

An important difference between the experiments of Ostrow (25) and McDonagh (19) and the present study was that the common bile duct was not cannulated. The enterohepatic circulation was intact in the

syringe. From the well mixed blood sample 0.5 ml was pipetted into two 2 ml glass tubes. From the first tube the whole blood hematocrit was measured in duplicate in 70 μ l capillary tubes.

To the second tube 5 μ l of 3% bovine albumin in isotonic saline was added and the samples equilibrated 30 min at 25°C. The sample was again thoroughly mixed by inversion and the hematocrit measured in duplicate to correct for changes in the packed cell and plasma volumes induced by the oncotic effect of the added albumin on red cell water.

The remainder of the blood in the tubes was then centrifuged and the bilirubin concentration of the plasma measured spectrophotometrically. The plasma was diluted in 0.1 M phosphate buffer (pH 7.4) and the difference in absorbance of the samples at 440 and 540 nm was measured. The difference in absorbance was divided

by the extinction coefficient of bilirubin ($\frac{\text{cmg}/100 \text{ ml}}{\text{cm}} =$

0.79) (23). Any increase in the plasma bilirubin from the blood samples to which albumin had been added was interpreted as derived from the erythrocytes.

Comparison of the mean results and standard deviations of the osmotic fragilities between pre- and post-phototherapy samples within $\mu\mu$ and J μ animals and between them was statistically analyzed by Fischer's exact test and by variance ratio (3, 7).

RESULTS

Table 1 records the measured values of body weight, blood hematocrits, plasma protein and bilirubin concentrations of the animals before and after 18 hours of continuous fluorescent irradiance.

All of the animals consistently lost weight but the blood hematocrits and plasma protein concentrations were not significantly changed. The reductions in plasma concentrations of bilirubin in the irradiated $\mu\mu$ animals were variable in that $\mu\mu_1$ and $\mu\mu_2$ exhibited reductions whereas the values in animals $\mu\mu_3$ and $\mu\mu_4$ before and after 18 hours of irradiance were not significantly different.

Table 2 records the immediate and delayed osmotic fragilities of the erythrocyte populations of the animals before and after irradiance. The data indicate 18 hours of irradiance by the fluorescent lamps had no influence on the immediate or delayed osmotic fragility of the erythrocyte populations in the J μ animals when exposed to progressively hypotonic solutions. By contrast the osmotic fragilities delayed hemolysis

were significantly greater in the blood samples from the jaundiced homozygous ($\mu\mu$) animals after the fluorescent irradiance. When statistically analyzed comparison of the variances (F test) of the delayed hemolysis in the $\mu\mu$ animals after the irradiation demonstrated a significantly greater population of erythrocytes which were more susceptible to osmotically induced hemolysis than before the 18 hours of irradiance. Analysis of the variance in hemolysis of erythrocytes from the $\mu\mu$ animals compared to the values of their non-jaundiced littermates showed an even greater difference in osmotic fragility. Of significance both biologically and statistically the $\mu\mu$ animals exhibited increased erythrocyte fragility after 18 hours of phototherapy whereas none of their non-jaundiced littermates. J μ showed an increased fragility. Indeed the mean values and standard deviations of the delayed fragilities in the J μ animals were smaller after 18 hours of fluorescent irradiation. Tabulation of the results in a 2x2 table indicated that all of the $\mu\mu$ animals exhibited increased osmotic fragility and none of their heterozygous littermates did so. By application of Fischer's exact test these results have a combined two-tailed probability value <0.029.

The mean bilirubin concentration of erythrocytes in the comparable aged $\mu\mu$ animals was 0.914 mg/100 ml of packed cells with a range of 0.1-1.9 which represented 10% of the mean plasma concentration of bilirubin in the 5 animals studied.

DISCUSSION

The animals were splenectomized before the irradiance studies in order to retard the rate of removal of light damaged cells from the circulation and enhance the possibility for their detection by an increased osmotic fragility.

Photosensitized hemolysis associated with bilirubin as with other photosensitizing hemolytic agents is thought to result from the photon induced injury to the erythrocyte

membrane and a secondary colloid osmotic swelling of the erythrocyte due to its contained hemoglobin (5). The membrane pump(s) can no longer maintain the concentration gradient between intra and extra erythrocyte sodium. The oncotic effect of hemoglobin increases the erythrocyte volume and spherocytosis occurs which precedes the actual hemolysis.

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An important difference between the experiments of Ostrow (25) and McDonagh (19) and the present study was that the common bile duct was not cannulated. The enterohepatic circulation was intact in the

animals of the current experiments. The latter circumstance would enhance the possibility of greater intestinal absorption of unconjugated bilirubin within the intestinal lumen consequent to greater biliary excretion associated with the fluorescent irradiance (15-27). Thus even though there may have been greater hepatic excretion of bilirubin during phototherapy the opportunity for its enterohepatic circulation was also increased.

In view of the well documented photosensitizing hemolytic effects of bilirubin in vitro on erythrocytes the present in vivo studies in jaundiced Gunn rats do suggest that photosensitized hemolysis may occur in infants who are treated by phototherapy. Whether a photosensitized hemolysis occurs depends in large measure on whether sufficient bilirubin concentrations are within the erythrocyte's membrane. Its relative rarity would indicate little erythrocyte bilirubin is present clinically at the time phototherapy is initiated.

ACKNOWLEDGEMENTS

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REFERENCES

- Blum H F. General photochemical aspects of photodynamic action. In *Photodynamic action and diseases caused by light*. American Chemical Society Monograph Series. Reinhold Publishing Co. New York 1941. p 47.
- Bratlad D. Reserve albumin binding capacity, salicylate saturation index and red cell binding of bilirubin in neonatal jaundice. *Arch Dis Child* 48: 393 1973.
- Brownlee K A. *Industrial experimentation*. Chemical Publishing Co. Brooklyn NY 1953. 4th ed. p 34.
- Cartwright G E. *Diagnostic laboratory hematology*. Grune and Stratton. New York 1968. 4th ed. p 290.
- Cook J S. The quantitative interrelationships between ion fluxes, cell swelling and radiation dose in ultraviolet hemolysis. *J Gen Physiol* 48: 719 1965.
- Crosby W H & Furth F W. A modification of the benzidine method for measurement of hemoglobin in plasma and urine. *Blood* 11: 380 1965.
- Dixon W J & Massey F J Jr. *Introduction to statistical analysis*. McGraw Hill. New York 1969. 3rd ed. p 243.
- Doleiden I H, Fahrenholtz S H, Lamola A A & Trozzolo A M. Reactivity of cholesterol and some fatty acids toward singlet oxygen. *Photochem Photobiol* 70: 519 1974.
- Girotti A W. Bilirubin sensitized photoinactivation of enzymes in the isolated membrane of the human erythrocyte. *Photochem Photobiol* 24: 575 1976.
- Girotti A W. Glyceraldehyde 3 phosphate dehydrogenase in the isolated human erythrocyte membrane: selective displacement by bilirubin. *Archiv Biochem Biophys* 173: 210 1976.
- Girotti A W. Photodynamic action of protoporphyrin IX on human erythrocytes: cross-linking of membrane proteins. *Biochem Biophys Res Commun* 72: 1367 1976.
- Goldstein B D & Harber L C. Erythropoietic photoporphyrin lipid peroxidation and red cell membrane damage associated with photohemolysis. *J Clin Invest* 51: 892 1972.
- Gornall A G, Bardavill C J & David M M. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 177: 751 1949.
- Howe H B, Hadjind C R & Engel R R. Effect of phototherapy on serum bilirubin levels and red blood cell survival in congenitally jaundiced Gunn rats. *J Lab Clin Med* 97: 221 1978.
- Lester R & Schmid R. Intestinal absorption of bile pigments. I. The enterohepatic circulation of bilirubin in the rat. *J Clin Invest* 47: 736 1963.
- John E. Complications of phototherapy in neonatal hyperbilirubinemia. *Aust Paediatr J* 11: 53 1975.
- Milloy H T & Evelyn K A. The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem* 119: 481 1937.
- Maurer H M, Frutkin M, McWilliams N B, Kirkpatrick B, Draper D, Higgins J C & Hunter C R. Effects of phototherapy on platelet counts in low birthweight infants and on platelet production and life span in rabbits. *Pediatrics* 57: 506 1976.
- McDonagh A F. The photochemistry and photometabolism of bilirubin. In G B Odell, R Schafer & A Simopoulos (eds). *Phototherapy in the Neonate*. An Overton. Natl Acad Sciences. Washington D C 1974. p 36.
- McDonagh A F. The role of singlet oxygen in bilirubin photooxidation. *Biochem Biophys Res Commun* 44: 1306 1971.
- Nilsson R, Scanbeck G & Wennersten G. Primary mechanisms of erythrocyte photolysis induced by biological sensitizers and phototoxic drugs. *Photochem Photobiol* 72: 183 1975.
- Odell G B, Brown R S & Kopelman A E. The photodynamic action of bilirubin on erythrocytes. *J Pediatr* 81: 473 1972.
- Odell G B, Cukier J O, Ostrea E M Jr, Magalang A C & Poland R L. The influence of fatty

- acids on the binding of bilirubin to albumin *J Lab Clin Med* 89 795 1977
- 4 Ostrea E M Jr & Odell G B Photosensitized shift in the O dissociation curve of fetal blood *Acta Paediatr Scand* 63 341 1974
 - 5 Ostrow J D Photocatabolism of labeled bilirubin in the congenitally jaundiced (Gunn) rat *J Clin Invest* 50 107 1971
 - 6 Patel D A Prides R S & Behrman R E Failure phototherapy to reduce serum bilirubin in newborn infants *J Pediatr* 77 1048 1970
 - 7 Poland E L & Odell G B Physiologic jaundice: the enterohepatic circulation of bilirubin *N Engl J Med* 284 1 1971
 - 8 Robinson S H & Schunior A Hemolytic anemia induced by light therapy in jaundiced rats *Proc Soc Biol Med* 158 81 1978
 - 9 Saeki K Studies in the photodynamic haemolytic action of bilirubin I Presence or absence of the photodynamic haemolytic action: an inquiry into the factor concerned in the after effects of the action and presence or absence of photodynamic haemolytic action biliverdin *Jap J Gastroenterol* 4 153 193
 - 10 Spikes J D Photodynamic action *In* A C Giese (ed) *Photophysiology* Academic Press New York 1968 vol 3 p 33
 - 11 Van Assendelft O W *Spectrophotometry of haemoglobin derivatives* Thomas Springfield Ill 1970 p 55
 - 12 Vogt T F Phototherapy of neonatal hyperbilirubinemia: bilirubin in unexposed areas of the skin *J Pediatr* 85 707 1974
 - 13 Windorfer A Jr Faxelius G & Boreus L O Studies on phototherapy in newborn infants. Influence on protein binding of bilirubin and salicylate and on activity of acetyl salicylic acid esterase *Acta Paediatr Scand* 64 293 1975
 - 14 Final report of the committee on Phototherapy in the Newborn Natl Acad Sciences Washington DC 1974

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REFERENCES

- Blum H F. General photochemical aspects of photodynamic action. In *Photodynamic action and diseases caused by light*. American Chemical Society Monograph Series. Reinhold Publishing Co. New York 1941 p 47.
- Bratlid D. Reserve albumin binding capacity, salicylate saturation index and red cell binding of bilirubin in neonatal jaundice. *Arch Dis Child* 48: 193 1973.
- Brownlee K A. *Industrial experimentation*. Chemical Publishing Co. Brooklyn NY 1953 4th ed p 34.
- Cartwright G E. *Diagnostic laboratory hematology*. Grune and Stratton New York 1968 4th ed p 290.
- Cook J S. The quantitative interrelationships between ion fluxes, cell swelling and radiation dose in ultraviolet hemolysis. *J Gen Physiol* 48: 719 1965.
- Crosby W H & Furth F W. A modification of the benzidine method for measurement of hemoglobin in plasma and urine. *Blood* 11: 380 1956.
- Dixon W J & Massey F J Jr. *Introduction to statistical analysis*. McGraw Hill New York 1969 3rd ed p 243.
- Doleiden F H, Fahrenholtz M M, Lamola A A & Trozzolo A M. Reactivity of cholesterol and some fatty acids toward singlet oxygen. *Photochem Photobiol* 20: 519 1974.
- Girotti A W. Bilirubin sensitized photoinactivation of enzymes in the isolated membrane of the human erythrocyte. *Photochem Photobiol* 24: 675 1976.
- Girotti A W. Glyceraldehyde 3-phosphate dehydrogenase in the isolated human erythrocyte membrane: selective displacement by bilirubin. *Arch Biochem Biophys* 173: 710 1976.
- Girotti A W. Photodynamic action of protoporphyrin IX on human erythrocytes: cross-linking of membrane proteins. *Biochem Biophys Res Commun* 72: 1167 1976.
- Goldstein B D & Harber L C. Erythropoietic photophosphorylation, lipid peroxidation and red cell membrane damage associated with photohemolysis. *J Clin Invest* 51: 897 1972.
- Gornall A G, Bardavill C J & David M M. Determination of serum proteins by means of the biuret reaction. *J Biol Chem* 177: 751 1949.
- Howe R B, Hadland C R & Ensel M R. Effect of phototherapy on serum bilirubin levels and red blood cell survival in congenitally jaundiced Gunn rats. *J Lab Clin Med* 97: 271 1978.
- Lester R & Schmid R. Intestinal absorption of bile pigments. I. The enterohepatic circulation of bilirubin in the rat. *J Clin Invest* 47: 736 1963.
- John E. Complications of phototherapy in neonatal hyperbilirubinemia. *Aust Paediatr J* 11: 53 1975.
- Milloy H T & Evelyn K A. The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem* 119: 481 1937.
- Maurer H M, Fritkin M, McWilliams N B, Kirkpatrick M, Draper D, Higgins J C & Hunter C R. Effects of phototherapy on platelet counts in low birthweight infants and on platelet production and life span in rabbits. *Pediatrics* 57: 506 1976.
- McDonagh A F. The photochemistry and photometabolism of bilirubin. In G B Odell, R Schafer & A Simopoulos (eds). *Phototherapy in the newborn: An Overview*. Natl Acad Sciences Washington DC 1974 p 56.
- McDonagh A F. The role of singlet oxygen in bilirubin photooxidation. *Biochem Biophys Res Commun* 44: 1306 1971.
- Nilsson R, Seimbeck G & Wennersten G. Primary mechanisms of erythrocyte photolysis induced in biological sensitizers and phototoxic drugs. *Photochem Photobiol* 22: 183 1975.
- Odell G B, Brown R E & Kopelman A E. The photodynamic action of bilirubin on erythrocytes. *J Pediatr* 81: 473 1972.
- Odell G B, Cukier J O, Ostrea E M Jr, Magla-lang A C & Poland M L. The influence of fatty

LETTER TO THE EDITOR

BENIGN PAROXYSMAL TORTICOLLIS IN INFANCY

Sir

I read with great interest the paper on Benign paroxysmal Torticollis in Infancy by Sanner & Bergstrom (4)

I completely agree that most of the differential diagnoses mentioned by the authors (posterior fossa tumor cervical dislocation epilepsy Sandifer's syndrome etc.) lack—among other symptoms—the regular periodicity of paroxysmal torticollis but I wish to comment on their differential diagnosis as to drug induced dystonic reactions

Paroxysmal torticollis is not the only condition which recurs with a regular periodicity Drug induced torticollis can also occur with a regular periodicity One of our patients (case 3)—reported in detail in our original paper—presented with a distressing and bizarre condition which we called cyclic torticollis (3) For more than a year this patient suffered from torticollis recurring every one to two months and lasting 2 to 4 days Observations on

two different occasions in a department of neurology did not elucidate the origin of his bizarre disease Sensitized by our previous cases we asked the mother whether her son had been given metoclopramide one of the drugs causing dystonic reactions We were then told that he suffered from cyclic vomiting and that whenever he started vomiting he received this drug A complete discontinuation of metoclopramide rescued the patient from his distressing symptoms¹

Drug induced dystonia does not necessarily involve the face and mouth Since publication of our paper in 1970 we have seen several patients in whom the paroxysmal torticollis was the only manifestation of drug induced dystonia

Table 1 (2) illustrates that torticollis can indeed be accompanied by trismus and facial grimacing but that these accompanying symptoms are not obligatory More frequently the

Table 1 Symptoms of drug induced dystonia

Symptoms	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10
Oculogyric crisis	+	+	+	+		+	+	+	+	+
Retrocollis	+	+	+	+	+	+	+			+
Torticollis	+		+	+		+	+		+	+
Neck pain	+	+	+	+		+				
Tremor		+				+	+	+		
Trismus		+				+	+			
Anxiety	+		+			+	+			+
Dysarthria			+	+			+			
Facial grimacing	+	+								+
Perspiration		+								+
Akathisia or motor restlessness	+					+	+			
Clonic movements of facial muscles neck or back	+					+			+	
Contractions of abdominal and lumbar muscles										
Dysphagia					+			+		
Pain in limbs				+						
Retropulsion							+			

Ref. Tijdschr Gastroenterol 14 05 1971

SHORT COMMUNICATION

DIAGNOSIS AND THERAPEUTIC STUDIES IN IDIOPATHIC PULMONARY HEMOSIDEROSIS

Idiopathic pulmonary hemosiderosis was diagnosed in two children who presented with marked iron deficiency anemia and were refractory to long term iron therapy. The importance of early diagnosis and the beneficial effect of prednisone is discussed.

Case I The patient was well until age 2½ years when fatigue, pallor, coughing and occasional hemoptysis appeared. On examination she was pale; there were no other abnormal findings. The hemoglobin concentration was only 4.8 g/l. Iron deficiency anemia was diagnosed and she was treated with blood transfusions and oral iron. One year later the symptoms recurred. The physical examination was unremarkable except for pallor. Chest radiograms showed infiltrations in both lung bases. The sputum contained iron-laden macrophages. Cow's milk hypersensitivity was ruled out by lack of improvement on a milk-free diet for about 2 months and a lack of serum precipitins to cow milk proteins. She was treated with prednisone (5 mg×4) daily for one and a half years and intermittently (30 mg weekly and 2½ mg every second day) thereafter until she died. ACTH (30 I.E. every 2 weeks and 4 weeks) was given for eight years. azathioprin (75 mg daily) was administered thereafter for two years. After eight years of disease at age 12 her lung function started to deteriorate. She died at the age of 16 years in respiratory failure after several episodes of hemoptysis. Autopsy showed marked pulmonary hemosiderosis and pulmonary hemorrhages.

Case II She was well until age 3 years when fatigue, irritability and episodes of coughing began. The hematologic findings were typical

of iron deficiency anemia and she was treated with oral and parenteral iron and blood transfusion with only temporary increase in hemoglobin concentration. Chest radiograms then showed widespread infiltrations. A needle aspiration of the lung was negative but iron-stained macrophages were demonstrated repeatedly in laryngeal swabs and gastric washings. Lung biopsy confirmed the diagnosis of pulmonary hemosiderosis. One gram of dry lung tissue contained 6.87 mg of iron. She was without cow's milk for about one year. No change of her condition was observed when cow's milk was reintroduced. Prednisone (5 mg×4) was given orally for one month and then 20 mg every other day. Half a year later the pulmonary symptoms and anemia reappeared. Daily dosage prednisone (5 mg×4) was reinstituted for a short period and then changed again to 20 mg every other day. Two years after the prednisone therapy was started she is thriving, growing well and has no signs of active disease.

Comments Only three cases of idiopathic pulmonary hemosiderosis have been reported from Scandinavia (3, 5, 7). The diagnosis may easily be missed and not made before autopsy (7, 8). Although iron therapy should be avoided because it can enhance the deposition of iron in the lung (1, 2, 6), many cases such as our own are initially treated with oral or parenteral iron or blood transfusions because of severe iron deficiency anemia (4, 5, 6). In cases of iron deficiency anemia with no response to oral iron and no apparent external blood loss, one must consider the possibility of an internal hemorrhage as found in pulmonary hemosiderosis. The most important

torticollis is accompanied by retrocollis and oculogyric crises—symptoms which are also mentioned in some of the cases reported by the authors.

Again I am puzzled by the fact that all four cases reported by Sanner & Bergstrom also present—besides the torticollis—some other symptoms of drug induced dystonia which are mentioned in Table 1: retrocollis (case 1) oculogyric crisis (case 2) tortipelvis (= curvature of the trunk in cases 2, 3 and 4) tremor (case 2) perspiration (case 4) and possibly dysphagia (case 2). For these reasons I wish to refer to my previous statement.

It is very important to stress that whenever a patient presents a paroxysmal or cyclic torticollis it can be a symptom of drug induced dystonia (1). The diagnosis of such reactions can be very difficult unless the physician has a high index of suspicion. A careful history should be taken every time one is confronted with this symptom searching for the dystonia inducer and enumerating these drugs for the parents (phenothiazines, metoclopramide, haloperidol, etc.). Indeed it has been our experience that some parents use some of these drugs regularly as a hypnotic as an anti-emetic or as a cough syrup for their children and no longer consider them as drugs.

I fully agree with the authors that paroxysmal torticollis remains a puzzling condition with many unsolved questions. Further careful observation, accurate description of all the accompanying symptoms and a detailed hi-

story taking could help in clarifying the entity of this syndrome.

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REFERENCES

- 1 Casteels van Daele M. Paroxysmal torticollis in infancy. *Am J Dis Child* 130: 88, 1970.
- 2 Casteels van Daele M & Jaeken J. Primperan and its side effects. *Tijdschr Gastroenterol* 14: 65, 1971.
- 3 Casteels van Daele M, Jaeken J, van der Schueren A, Zimmerman A & van den Bon P. Dystonic reactions in children caused by metoclopramide. *Arch Dis Child* 45: 130, 1970.
- 4 Sanner G & Bergstrom B. Benign paroxysmal torticollis in infancy. *Acta Paediatr Scand* 68: 719, 1979.

The Editor has asked Dr Sanner to comment on this letter.

Sir,

It seems quite clear that according to the experience of Casteels Van Daele, drug induced dystonia must always be held in mind in cases with torticollis, even in the absence of facial involvement. However, the prescription of phenothiazine drugs for small children—and especially infants—seems to be far less frequent in Sweden than in some other countries.

Our four patients had not been given any drugs.

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CASE REPORT

PRIMARY MYELOPEROXIDASE DEFICIENCY ASSOCIATED WITH IMPAIRED NEUTROPHIL MARGINATION AND CHEMOTAXIS

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ABSTRACT Robertson C F Thong Y H Hodge G L and Cheney K (Department of Paediatrics University of Adelaide and Department of Haematology The Adelaide Children's Hospital Adelaide South Australia) Primary myeloperoxidase deficiency associated with impaired neutrophil margination and chemotaxis *Acta Paediatr Scand* 68 915 1979 —The first infant with myeloperoxidase deficiency is described. Impairment of neutrophil margination and chemotaxis may have contributed to recurrent infections.

KEY WORDS Myeloperoxidase deficiency chemotaxis neutrophil leucocytes

Primary deficiency of myeloperoxidase (MPO) is a very rare disorder of phagocytic cells first described by Alius & Alius (27) as a coincidental finding in a healthy adult female: the levels of the enzyme in her parents, sister and four children were all normal. In 1963 Grig naschi (8) reported MPO deficiency in two asymptomatic siblings: one of these had two children with normal enzyme levels. Stendahl (20) described the deficiency in a 46-year-old man who presented with pustular psoriasis; this patient had an identical twin who also had pustular psoriasis but died from myocardial infarction prior to the study. Lehrers (13) patient was a 49 year-old man who suffered from chronic bronchitis, maturity-onset diabetes mellitus and systemic candidiasis.

We report here the first case of MPO deficiency diagnosed in infancy. This patient presented with a history of repeated infections. The MPO deficiency by itself cannot account entirely for the undue susceptibility to infection. Although the interaction between MPO and hydrogen peroxide (H_2O_2) and halide is considered by Klebanoff to be a potent microbicidal mechanism of phagocytic cells (12)

this conclusion is not supported by recent studies (10). Moreover, most patients with MPO deficiency do not demonstrate a strong predilection towards infectious complications and their neutrophils have only a slight delay in *in vitro* killing of microorganisms. Hence immunological studies were conducted to determine whether an associated immunodeficiency was responsible for this infant's clinical presentation.

CASE REPORT

The patient M L T is a white male, now 19 months old. He was born at term after an uneventful pregnancy with a weight of 3000 g. He developed a candida perineal infection at age 3 months; this required more than a month of nystatin treatment to clear. At age 6 months he had bronchitis. Recurrent aphthous ulceration began at this time. These were localised to the lateral edges and tip of the tongue, with occasional ones on the buccal mucosa. They recurred every 3-4 weeks and persisted for 2-3 weeks before healing with scar formation. There were 3 episodes of purulent conjunctivitis from age 9-17 months: a swab was taken on one occasion and this grew *Haemophilus influenzae* type b. He also had otitis externa at age 10 months and otitis media at age 11 months.

There was no family history of increased susceptibility to infection. The parents were not consanguineous. The mother aged 6 years and father aged 4 years were

point in the management of a patient with pulmonary hemosiderosis appears to be early diagnosis and the avoidance of iron therapy and blood transfusions and the use of corticosteroid therapy. Short term courses of steroids during the active stage of the disease seem to improve these patients (8). It is doubtful, however, whether longterm steroid therapy is of value, as our first case demonstrated.

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REFERENCES

- 1 Donald K. J., Edwards R. L. & McEvoy J. D. S. Alveolar capillary basement membrane lesions in Goodpasture's syndrome and idiopathic pulmonary hemosiderosis. *Am J Med* 59: 647, 1975.
- 2 Donlan C. J., Srodes H. C. & Duffy F. D. Idiopathic pulmonary hemosiderosis. *Chest* 68: 577, 1975.
- 3 Gellerstedt N. Über die essentielle anaemische Form der braunen Lungeninduration. *Acta Pathol Scand* 16: 386, 1939.
- 4 Gillmann P. A. & Zinkman W. H. Severe idiopathic pulmonary hemosiderosis in the absence of clinical or radiologic evidence of pulmonary disease. *J Pediatr* 75: 118, 1963.
- 5 Halvorsen S. Cortisone treatment of idiopathic pulmonary hemosiderosis. *Acta Paediatr Scand* 45: 119, 1956.
- 6 Hyatt W. W., Adelstein E. R., Halazum J. F. & Lukens J. N. Ultrastructure of the lung in idiopathic pulmonary hemosiderosis. *Am J Med* 47: 877, 1973.
- 7 Jacobsen K. B. Plutselig død ved idiopatisk pulmonal hemosiderose. *Nord Med* 86: 974, 1971.
- 8 Soergel K. H. & Sommers S. C. Idiopathic pulmonary hemosiderosis and related syndromes. *Am J Med* 33: 499, 1962.

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REFERENCES

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- 5 Hultverson B. Cortisone treatment of idiopathic pulmonary hemosiderosis. *Acta Paediatr Scand* 45: 189 (1956).
- 6 Hyatt R W, Adelstein E R, Halazum J F & Lukens J N. Ultrastructure of the lung in idiopathic pulmonary hemosiderosis. *Am J Med* 57: 877 (1974).
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Table 1 Immunological screening tests on an infant with myeloperoxidase deficiency

Test	Results	Normal range
Neutrophil function		
Marrow pool	3 345/ μ l increase	>2 000
Margination pool	22 7% increase	>45%
Bactericidal	87 5%	
	(<i>S. aureus</i> killed in 2 h)	70-90
Fungicidal	72 0%	
	(<i>T. labrata</i> killed in 4 h)	75-90%
Hexose monophosphate shunt activity	59	
	(stimulation index)	>3.0
Quantitative iodination	1.2	
	(pmols/ 10^7 cells/h)	>1.0
Chemotaxis	0.61 \pm 0.33	1.26 \pm 0.07
Serum immunoglobulins		
IgG	7.5 g/l	2.6-13.9
IgA	0.45 g/l	0.7-1.2
IgM	0.45 g/l	0.3-1.5
IgE	3.4 U/ml	64-392
Serum complement		
C3	1.74 g/l	0.65-1.40
C4	0.29 g/l	0.70-0.40
CH50	128 U/ml	95-165
T-cells	68%	40-70
B-cells	11%	5-15
Lymphocyte transformation*		
Phytohemagglutinin	41 498 cpm	>10 000
Pokeweed mitogen	13 658 cpm	>5 000
Concanavalin A	29 907 cpm	>10 000

Mean \pm S.D. of 4 experiments ($p < 0.01$) on 4 different occasions

* Expressed as counts per minute (cpm) 3 H thymidine uptake in stimulated cultures resting cultures showed uptake of 519 cpm

healthy. The only sibling, a male aged 4 years, was also healthy.

Physical examination at age 12 months revealed a well nourished infant weighing 9.85 kg (25-50%) and measuring 73.0 cm in height (25-50%). Development milestones were normal. The haemoglobin was 10.5 g/dl, platelet count $150\,000/\mu\text{l}$ and white cell count $11\,600/\mu\text{l}$ with 29% neutrophils, 1% eosinophils, 68% lymphocytes, 2% monocytes. The patient was admitted to the Adelaide Children's Hospital at 12 months of age for immunological investigations; during this time he was clinically well and free from infection.

MATERIALS AND METHODS

In order to exclude cyclic neutropenia, absolute neutrophil counts were performed twice weekly over 6 weeks. The adequacy of the marrow neutrophil reserve was tested by doing an absolute neutrophil count by finger prick at 0 and 5 hours after intravenous injection of 50 mg hydrocortisone (5). The neutrophil margination pool was assessed at 0 and 1 hour after the subcutaneous injection of 0.1 ml 1:1000 solution of adrenaline (18).

The serum immunoglobulins (A, G, M) and complement (C3, C4) concentrations were measured by radial immunodiffusion using commercial plates (Behringwerke, W.

Germany), serum IgE by the Phadebas test kit (Pharmacia, Sweden), total haemolytic complement by the lysis of sheep red cells (15).

Lymphocytes and neutrophils were purified from heparinized blood by a one step gradient sedimentation procedure.

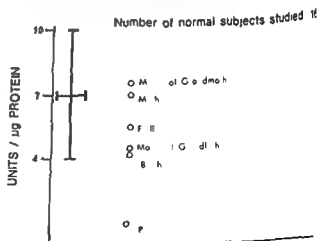


Fig. 1 Quantitative assay of myeloperoxidase activity in leukocytes.



Fig 2 Peripheral blood smear of patient showing positive staining for myeloperoxidase in eosinophil (A) but not neutrophil (B)

dures (6) T-cells enumerated by spontaneous rosetting with sheep red cells (19) lymphocyte transformation to mitogens by a micro-culture method (4) neutrophil hexose-monophosphate shunt activity by conversion of ^3H 1-glucose to ^3H CO_2 (3) quantitative iodination by a microassay technique (6) bactericidal capacity by reduction of *Staphylococcus aureus* colony counts (21) fungicidal capacity by reduction of *Torulopsis glabrata* colony counts () and chemotaxis by migration under agarose (5).

White blood cells were stained for peroxidase by a modification of the method of Goodpasture (7) using toluidine as substrate. Myeloperoxidase activity was assayed by the method of Klebanoff (11) with slight modification (13) using α -naphthylamine as substrate.

RESULTS

Twice weekly white cell counts over 6 weeks showed that absolute neutrophil numbers were consistently over $2000/\mu\text{l}$ thereby excluding the possibility of cyclic neutropenia. The results of immunological screening tests are presented in Table 1. All tests were normal

except for impaired neutrophil margination, chemotaxis and quantitative iodination.

Quantitative studies revealed normal MPO concentrations in family members (Fig. 1). Peripheral blood smear of the patient (Fig. 2) confirmed the deficiency of MPO in his neutrophils but not eosinophils. Family studies showed that brother and mother have normal neutrophil iodination (3.4 and 3.7 pmols/ 10^7 cells/h respectively) and chemotaxis (0.9 and 1.15 mm/2 h respectively). When neutrophil bactericidal and fungicidal assays were performed at varying incubation times, a delay in killing of fungi (Fig. 3) but not bacteria (Fig. 4) was observed compared to other family members and controls.

DISCUSSION

Neutrophils are important mediators of resistance to infection (2, 3). They are the first cells

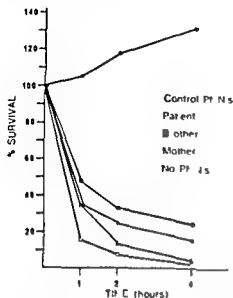


Fig. 3 Fungicidal capacity of neutrophils in relation to incubation time

to gather at the site of inflammation where they ingest and kill microorganisms. The process involves mobilization of these cells from marrow stores, the deployment to the lining of the blood vessels (margination pool) and their final egress into tissues by means of chemotaxis. It has been shown that two to four hours after bacterial invasion is the most critical period for the inflammatory response to control infection (16). Hence patients with chemotactic disorders are invariably subjected to infectious complications (2, 3, 28). The results of the present studies indicate the impairment of neutrophil margination and chemotaxis may be responsible for the recurrent infections in our patient. The associated MPO deficiency may also be contributory to his problems, although this conclusion must be tentative in the light of recent findings that the MPO-H₂O halide system is not directly microbicidal. Others (17) have shown that iodination of microbial membranes can be inhibited by ascorbate without a concomitant decrease in killing. Moreover, addition of superoxide dismutase increases iodination but decreases killing (1, 9). In vitro studies have shown only a slight delay in the killing of microorganisms (13) and this is confirmed by our data for fungi but not bacteria. Thus the MPO-H₂O halide system may play only a

minor role to the other microbicidal mechanisms such as superoxide anion cationic proteins, lysozyme and lactoferrin (12).

Secondary MPO deficiency has been described in association with haematological disorders (4, 14) but their infectious complications have been attributed to MPO deficiency rather than debilitation and immunodepression. Of the 6 cases of primary MPO deficiency previously reported, only 2 developed infectious complications in adulthood. One had diabetes mellitus and systemic candidiasis (13) while the other had pustular psoriasis (20). In the latter situation, the dermatological condition might have been the predisposing cause of the skin infection. It would appear that MPO deficiency per se may not predispose to recurrent infection. Our patient is unique in presenting with recurrent infections in infancy and this can be explained on the basis of a defect in neutrophil margination and chemotaxis.

The MPO enzyme is present in a wide variety of animal species (12). Several isoenzymes are known to exist. The enzyme of neutrophils and monocytes is different from that in eosinophils; hence the positive staining for MPO in eosinophils of our patient also

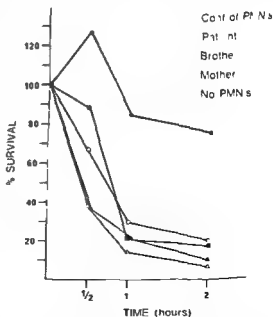


Fig. 4 Bactericidal capacity of neutrophils in relation to incubation time

though MPO deficiency in neutrophils has been demonstrated by quantitative iodination enzyme assay and direct staining. The gene for neutrophil MPO deficiency is believed to be transmitted by an autosomal recessive mode. The heterozygous state cannot be diagnosed by enzyme assay because parents do not show intermediate values.

REFERENCES

- Baehner R L, Murrmann S K, Davis J & Johnston R B Jr. The role of superoxide anion and hydrogen peroxide in phagocytosis associated oxidative metabolic reactions. *J Clin Invest* 56: 571 1975.
- Bellanti J A & Dayton D H. *The phagocytic cell in host resistance*. Raven Press, New York 1975.
- Bjorksten B & Que P G. Abnormalities of circulating phagocyte function. In R A Thompson (ed.) *Recent advances in clinical immunology*. Churchill Livingstone, Edinburgh 1975 vol 1 p 187.
- Davies A T, Brunning R T & Que P G. Polymorphonuclear leukocyte myeloperoxidase deficiency in a patient with myelomonocytic leukaemia. *N Engl J Med* 285: 789 1971.
- Dienard A S, Fortuny I M, Theohgides A, Anderson J L, Boer J & Kennedy H J. Studies on the neutropenia of cancer chemotherapy. *Cancer* 33: 1710 1974.
- Ferrante A & Thong Y H. A rapid one step procedure for purification of mononuclear and polymorphonuclear leukocytes from human blood using a modification of the hypaqueficoll technique. *J Immunol Methods* 24: 389 1978.
- Goodpasture E W. A peroxidase reaction with sodium nitroprusside and benzidine in blood smears and tissues. *J Lab Clin Med* 4: 44 1919.
- Grunaschi V J, Sperferato A M & Etcheverry M J. Un nuevo cuadro citocimico negatividad espontanea de las reacciones de peroxidadas oxidas y lipidio en la progenie neutrofila en los monocitos de dos hermanas. *Rev Assoc Med Argent* 77: 18 1971.
- Johnston R B Jr, Keele BB Jr, Misra H P, Lehmeyer J E, Webb L S, Baehner H L & Rajagopalan K V. The role of superoxide anion generation in phagocytic bactericidal activity. *J Clin Invest* 55: 1357 1975.
- Johnson H Jr. Oxygen metabolism and the microbicidal activity of macrophages. *Federation Proceedings* 37: 759 1978.
- Klebanoff S J. Inactivation of estrogen by rat uterine preparation. *Endocrinology* 76: 301 1965.
- Klebanoff S J. Antimicrobial systems in neutrophils: polymorphonuclear leukocytes. *Semin Hematol* 11: 117 1975.
- Lehrer R I & Cline M J. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to candida infection. *J Clin Invest* 48: 1478 1969.
- Lehrer R I, Goldberg L S, Apple M & Rosenthal N P. Refractory megaloblastic anemia with myeloperoxidase deficient neutrophils. *Ann Int Med* 76: 447 1972.
- Mayer M M. Complement and complement fixation. In E A Kabat (ed.) *Experimental immunology*. Charles C Thomas, Springfield, Ill 1971 2nd ed p 133.
- Miles A Z, Miles E M & Burke J. The value and duration of defence reactions of the skin to primary lodgement of bacteria. *Br J Exp Pathol* 38: 79 1957.
- McColl C E, DeChatelet L R, Cooper M R & Ashburn P. The effect of ascorbic acid on bactericidal mechanisms of neutrophils. *J Infect Dis* 124: 194 1971.
- Samuels A J. Primary and secondary leukocyte changes following the intramuscular injection of epinephrine hydrochloride. *J Clin Invest* 30: 941 1951.
- Scheinberg M, Blacklow N R, Goldstein A L, Parnino J A, Rose F B & Cathcart E S. In fluenza response of T-cell lymphopenia to thymosin. *N Engl J Med* 294: 1708 1976.
- Siendahl O & Lundgren S. Function of granulocytes with deficient myeloperoxidase mediated iodination in a patient with generalized pustular psoriasis. *Scand J Haematol* 16: 144 1976.
- Thong Y H, Ferrante A & Ness H. Neutrophil phagocytic and bactericidal dysfunction induced by bilirubin. *Aust Paediatr J* 13: 787 1977.
- Thong Y H & Ferrante A. Phagocytosis and killing of *Torulopsis glabrata* by human neutrophils. *IRCS Med Sci* 5: 487 1977.
- Thong Y H & Rencis V. Bilirubin inhibits hexose monophosphate shunt activity of phagocytosing neutrophils. *Acta Paediatr Scand* 66: 757 1977.
- Thong Y H & Ferrante A. Inhibition of mitogen induced human lymphocyte proliferative responses by quinine. *Am J Trop Med Hyg* 27: 354 1978.
- Thong Y H, Douglas B S & Ferrante A. Abnormal neutrophil chemotaxis in a syndrome of unusual facies, proportionate small stature and sensorineural deafness: mucopolysaccharidosis. *Acta Paediatr Scand* 67: 383 1978.
- Thong Y H & Ferrante A. A semi automated microassay technique for quantitative neutrophil iodination. *J Immunol Methods* 20: 97 1978.
- Undritz E. Die Alus-Grunaschi Anomalie der erblich konstitutionelle Peroxydasedefekt der Neutrophilen und Monozyten. *Blut* 14: 179 1966.
- Ward P A. Leukotaxis and leukotactic disorders. A review. *Am J Pathol* 77: 570 1974.

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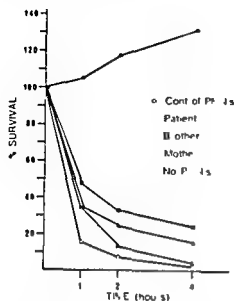


FIG. 3. Fungicidal capacity of neutrophils in relation to incubation time.

to gather at the site of inflammation where they ingest and kill microorganisms. The process involves mobilization of these cells from marrow stores, the deployment to the lining of the blood vessels (margination pool) and their final egress into tissues by means of chemotaxis. It has been shown that two to four hours after bacterial invasion is the most critical period for the inflammatory response to control infection (16). Hence patients with chemotactic disorders are invariably subjected to infectious complications (2, 3, 28). The results of the present studies indicate the impairment of neutrophil margination and chemotaxis may be responsible for the recurrent infections in our patient. The associated MPO deficiency may also be contributory to his problems, although this conclusion must be tentative in the light of recent findings that the MPO-H₂O₂ halide system is not directly microbicidal. Others (17) have shown that iodination of microbial membranes can be inhibited by ascorbate without a concomitant decrease in killing. Moreover, addition of superoxide dismutase increases iodination but decreases killing (1, 9). In vitro studies have shown only a slight delay in the killing of microorganisms (13) and this is confirmed by our data for fungi but not bacteria. Thus the MPO-H₂O₂ halide system may play only a

minor role to the other microbicidal mechanisms such as superoxide anion, cationic proteins, lysozyme and lactoferrin (12).

Secondary MPO deficiency has been described in association with haematological disorders (4, 14) but their infectious complications have been attributed to MPO deficiency rather than debilitation and immunodepression. Of the 6 cases of primary MPO deficiency previously reported, only 2 developed infectious complications in adulthood. One had diabetes mellitus and systemic candidiasis (13) while the other had pustular psoriasis (20). In the latter situation, the dermatological condition might have been the predisposing cause of the skin infection. It would appear that MPO deficiency *per se* may not predispose to recurrent infection. Our patient is unique in presenting with recurrent infections in infancy and this can be explained on the basis of a defect in neutrophil margination and chemotaxis.

The MPO enzyme is present in a wide variety of animal species (12). Several iso-enzymes are known to exist. The enzyme of neutrophils and monocytes is different from that in eosinophils; hence the positive staining for MPO in eosinophils of our patient al-

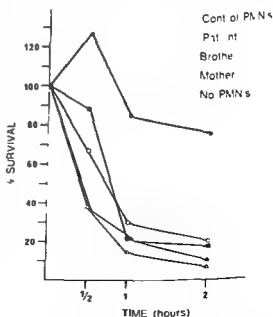


FIG. 4. Bactericidal capacity of neutrophils in relation to incubation time.

though MPO deficiency in neutrophils has been demonstrated by quantitative iodination enzyme assay and direct staining. The gene for neutrophil MPO deficiency is believed to be transmitted by an autosomal recessive mode. The heterozygous state cannot be diagnosed by enzyme assay because parents do not show intermediate values.

REFERENCES

- Baehner R L, Murrmann S K, Davis J & Johnston R B Jr. The role of superoxide anion and hydrogen peroxide in phagocytosis associated oxidative metabolic reactions. *J Clin Invest* 56: 571 (1975).
- Bellanti J A & Dayton D H. *The phagocytic cell in host resistance*. Raven Press, New York (1975).
- Bjorksten B & Quie P G. Abnormalities of circulating phagocyte function. In R A Thompson (ed.) *Recent advances in clinical immunology*. Churchill Livingstone, Edinburgh (1978) vol 1 p 187.
- Davies A T, Brunning R T & Quie P G. Polymorphonuclear leukocyte myeloperoxidase deficiency in a patient with myelomonocytic leukaemia. *N Engl J Med* 285: 789 (1971).
- Dienard A S, Fortuny I F, Theoligides A, Anderson G L, Boer J & Kennedy B J. Studies on the neutropenia of cancer chemotherapy. *Cancer* 33: 1710 (1974).
- Ferrante A & Thong Y H. A rapid one step procedure for purification of mononuclear and polymorphonuclear leukocytes from human blood using a modification of the hypaqueficoll technique. *J Immunol Methods* 24: 389 (1978).
- Goodpasture E W. A peroxidase reaction with sodium nitroprusside and benzidine in blood smears and tissue. *J Lab Clin Med* 4: 447 (1919).
- Grignaschi V J, Sperperato A M & Etcheverry M J. Un nuevo cuadro citquimico negatividad espontanea de las reacciones de peroxidadas oxidadas y lido en la progenie neutrofila y en los monocitos de dos hermanos. *Re Assoc Med Argent* 77: 18 (1971).
- Johnston R B Jr, Keele BB Jr, Misra H P, Lehmyer J E, Webb L S, Baehner R L & Rajagopalan K V. The role of superoxide anion generation in phagocytic bactericidal activity. *J Clin Invest* 55: 1357 (1975).
- Johnson R B Jr. Oxygen metabolism and the microbicidal activity of macrophages. *Federal Proceedings* 37: 7749 (1978).
- Klebanoff S J. Inactivation of estrogen by rat uterine preparation. *Endocrinology* 76: 301 (1965).
- Klebanoff S J. Antimicrobial systems in neutrophilic polymorphonuclear leukocytes. *Semin Hematol* 12: 117 (1975).
- Lehrer R I & Cline M J. Leukocyte myeloperoxidase deficiency and disseminated candidiasis: the role of myeloperoxidase in resistance to candida infection. *J Clin Invest* 48: 1478 (1969).
- Lehrer R I, Goldberg L M, Apple M & Rowenthal W P. Refractory megaloblastic anemia with myeloperoxidase deficient neutrophils. *Ann Int Med* 76: 447 (1972).
- Mayer M M. Complement and complement fixation. In E A Kabat (ed.) *Experimental immunology*. Charles C Thomas, Springfield Ill (1971) 2nd ed p 133.
- Miles A Z, Miles E M & Burke J. The value and duration of defence reactions of the skin to primary lodgement of bacteria. *Br J Exp Pathol* 38: 79 (1957).
- McColl C M, DeChatelet L R, Cooper M R & Ashburn P. The effect of ascorbic acid on bactericidal mechanisms of neutrophils. *J Infect Dis* 124: 194 (1971).
- Samuels A J. Primary and secondary leukocyte changes following the intramuscular injection of epinephrine hydrochloride. *J Clin Invest* 30: 941 (1951).
- Scheinberg M, Blacklow N R, Goldstein A L, Parnio J A, Rose F B & Cathcart E S. In fluenza response of T-cell lymphopenia to thymosin. *N Engl J Med* 294: 1708 (1976).
- Stendahl O & Lundgren S. Function of granulocytes with deficient myeloperoxidase mediated iodination in a patient with generalized pustular proriasis. *Scand J Haematol* 16: 144 (1976).
- Thong Y H, Ferrante A & Ness D. Neutrophil phagocytic and bactericidal dysfunction induced by bilirubin. *Aust Paediatr J* 13: 87 (1977).
- Thong Y H & Ferrante A. Phagocytosis and killing of *Torulopsis glabrata* by human neutrophils. *IRCS World Sci J* 5: 487 (1977).
- Thong Y H & Rencis V. Bilirubin inhibits hexose monophosphate shunt activity of phagocytosing neutrophils. *Acta Paediatr Scand* 66: 757 (1977).
- Thong Y H & Ferrante A. Inhibition of mitogen induced human lymphocyte proliferative responses by quinine. *Am J Trop Med Hyg* 27: 354 (1978).
- Thong Y H, Doudas B S & Ferrante A. Abnormal neutrophil chemotaxis in a syndrome of unusual facies, proportionate small stature and sensorineural deafness mutism. *Acta Paediatr Scand* 67: 383 (1978).
- Thong Y H & Ferrante A. A semi automated microassay technique for quantitative neutrophil iodination. *J Immunol Methods* 60: 797 (1978).
- Undritz E. Die Albus-Gnignaschi Anomalie der erblichkonstitutionelle Peroxydasedefekt der Neutrophilen und Monozyten. *Blut* 14: 179 (1966).
- Ward P A. Leukotaxis and leukotactic disorders. A review. *Am J Pathol* 77: 570 (1974).

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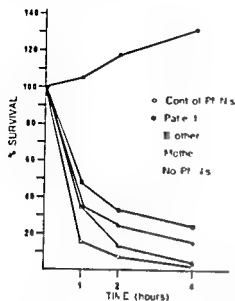


Fig. 3 Fungicidal capacity of neutrophils in relation to incubation time

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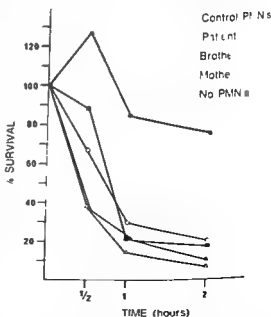


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CASE REPORT

HEMIHYPERTROPHY WITH UNILATERAL FOLLICULITIS AND ACNE

P KAAPA and ■ SUSITAIVAL

From the Departments of Paediatrics and Dermatology, Central Hospital of North Karelia, Joensuu, Finland

ABSTRACT Kaapa P and Susitaival P (Departments of Paediatrics and Dermatology, Central Hospital of North Karelia, Joensuu, Finland) Hemihypertrophy with unilateral folliculitis and acne. *Acta Paediatr Scand* 68 921 1979.—A case of congenital hemihypertrophy, right sided folliculitis and acne in a 16-year-old boy is described. Except for hemihypertrophy and skin changes on the right side, no other abnormalities were found. A similar combination could not be found in the literature.

KEY WORDS Hemihypertrophy, folliculitis, acne.

Hemihypertrophy is a rare congenital developmental anomaly, a unilateral enlargement of one half of the body, either complete or partial. Its etiology is not known.

Ringrose et al. (6) reported changes in skin colour, texture, secretion and temperature with unusual naevi and teleangiectasiae in 47% of their cases of hemihypertrophy. Mental deficiency is found in 28% of the cases. There is also a high correlation between renal (Wilms's) (1, 2, 4), adrenal (5) and hepatic (3) tumours and hemihypertrophy. We report a case of congenital hemihypertrophy with acne and folliculitis limited to the hypertrophic side.

CASE REPORT

A 16-year-old boy was seen by us because of folliculitis in the scalp and acne in the upper parts of the trunk, both on the right side only. According to the patient's history, his birth was normal after an uneventful pregnancy. His birth weight was 4450 g and the Apgar score 10/10. The boy's development was normal. At the age of 3 months the mother noticed a difference in temperature between the boy's hands, the right being warmer than the left. The difference in size between the two sides of his body was noticed later, but did not receive much attention.

The patient's uncle is said to have large hands and one side of the thorax more elevated, but we lack further evidence of hemihypertrophy in his case. The other members of the family are healthy.

Physical examination revealed a healthy school boy who measured 180.5 cm in height and 63 kg in weight,

being at the eighty-fourth percentile in the height chart and at the fiftieth in the weight chart for Finnish boys. The right side of the body was darker in colour and felt warmer than the left, even the superficial veins were more prominent to the right, especially on the right ankle. Clinically there were no signs of an AV shunt. The forehead and the cheekbones were more prominent on the right side and the right shoulder was elevated. The extremities measured 1 cm more in length on the right side. The maximum difference in circumference of the two sides was 1.5 cm (biceps and thigh). These differences were also demonstrable in radiographs of the extremities. Both sides showed a retardation of 2 to 3 years in bone age, slightly more on the left side. The boy's pubertal stage was expressed as G5 and P5 according to Tanner (1967).

On the right side of the patient's head there was mild folliculitis in two areas, one over the ear and the other extending from the forehead over the scalp. Both areas were constant and about 2 cm broad. There was also some acne with multiple comedones and a mild inflammatory reaction on the right upper thorax. New lesions were erupting continuously.

DISCUSSION

In the present case the only abnormalities were hemihypertrophy with accompanying skin changes. Acne and folliculitis were the boy's chief complaints. The enlargement of his right arm and leg, though previously noted, had not been considered severe enough for consulting a doctor. Acne was noticed at the age of 14 and is, though one-sided, a problem for the patient. The peculiar arrangement of

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Heredity is suggested in 7 published cases (2-7) hemihypertrophy occurring mostly in successive generations on the maternal side but this could not be demonstrated in the present case.

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CASE REPORT

REFLEX SYMPATHETIC DYSTROPHY IN TWO YOUNG FEMALES

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From the Departments of Paediatrics and Surgery, Karnsjukhuset Skövde, Sweden

ABSTRACT Wettrell G, Hallböök T and Hultquist C (Departments of Paediatrics and Surgery, Karnsjukhuset Skövde, Sweden). Reflex sympathetic dystrophy in two young females. *Acta Paediatr Scand* 68: 923-924, 1979. — Reflex sympathetic dystrophy in paediatric patients is a rarely recognized pain syndrome probably of neurovascular origin. The manifestations in two young females consisted of disabling pain and localized hyperesthesia in lower extremities without evident trauma. Sympathetic block followed by active mobilization and in the patient with atrophic changes, lumbar sympathectomy resulted in complete recovery. Reflex sympathetic dystrophy should be considered in the differential diagnosis of pain and tenderness in an extremity.

KEY WORDS Pain, extremity, children.

The Reflex Sympathetic Dystrophy syndrome (RSD) is characterized by persistent pain in an extremity. It involves mainly hyperaesthesia but vasomotor symptoms and atrophic changes may arise as well (2-7). The condition is often disabling and seems out of proportion when compared with the negligible trauma. The syndrome has attracted little attention in clinical paediatric practice and can be misdiagnosed resulting in severe disability (1, 3, 4, 5, 6).

This paper describes 2 girls with RSD who were successfully treated with sympathetic blocks. In one patient followed by sympathectomy.

CASE REPORTS

Case 1

A 14-year-old girl was first seen in the paediatric clinic after having suffered from pain in her right foot for 8 months. The pain had started after a period of intense physical activity and immobilization with plaster and analgetics had been futile. Physical examination revealed continuous pain up to the level of the malleoli and she could not set weight on her foot. There had been some swelling of the foot at first but now atrophy with hyper-

keratotic skin and brittle nails dominated. She complained of tenderness on the distal plantar surface and even more so localized to a point on the dorsum of the foot. The circumference of the right calf was 3 cm less than the left. Deep tendon reflexes and peripheral pulses were equal bilaterally.

An X-ray of the affected foot showed an aseptic partial necrosis in the head of the third metatarsal bone as well as a generalized and marked osteoporosis. Measurement of blood flow in the right calf by means of strain gauge occlusion plethysmography showed a slight decrease compared with the left side. The blood pressures measured in the ankles were equal and thermography showed no significant difference between sides. Laboratory results revealed normal sedimentation rate and serum protein electrophoresis.

A lumbar sympathetic block with 0.25% bupivacaine hydrochloride was performed with prompt but incomplete and temporary relief. Six days later a right lumbar sympathectomy was done and postoperatively the pain disappeared. Thermography showed the right lower leg and foot to be warmer than the left. After 9 days of intense physical therapy she was discharged painfree and within a few months she resumed full physical activity. The X-ray pictures normalized. No recurrence of pain has been noted during 1½ year follow-up.

Case 2

A 9-year-old girl came to the paediatric clinic with a 9-month history of pain localized to the right heel. Initially the pain had been intermittent but after 3 months it became continuous. For a short period there had been a slight swelling of the foot but no cyanosis. For the last

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- 1 Bjorklund S I Hemihypertrophy and Wilms's tumour. *Acta Paediatr Scand* 44: 287 1955
- 2 Fraumeni J F Jr Geiser C F & Manning M D Wilms tumor and congenital hemihypertrophy. Report of five new cases and review of literature. *Pediatrics* 40: 886 1967
- 3 Geiser C F Biez A Schindler A M & Shih V I Epithelial hepatoblastoma associated with con-

genital hemihypertrophy and cystathionuria. Presentation of a case. *Pediatrics* 46: 66 1970

- 4 Miller R W Fraumeni J F & Manning M D Association of Wilms's tumor with aniridia hemihypertrophy and other congenital malformations. *N Engl J Med* 270: 922 1964
- 5 Riedel H A Adrenogenital syndrome in a male child due to adrenocortical tumor. *Pediatrics* 10: 19 1955
- 6 Ringrose R F Jabbour J T & Keele D K Hemihypertrophy. *Pediatrics* 36: 434 1965
- 7 Scott A J Hemihypertrophy. Report of four cases. *J Pediatr* 6: 650 1935

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CASE REPORT

SYSTEMIC AIR EMBOLISM

A Possible Complication of Artificial Ventilation

C E BLANCO L A C RIETVELD and J H RUYS

*From the Department of Neonatology and the Department of Radiology
University Hospital Leiden The Netherlands*

ABSTRACT Blanco C E Rietveld L A C and Ruys J H (Department of Neonatology and Department of Radiology University Hospital Leiden The Netherlands) Systemic air embolism. A possible complication of artificial ventilation. *Acta Paediatr Scand* 68: 925-927, 1979. —A baby of 1100 g and a gestational age of 28 weeks with severe idiopathic respiratory distress syndrome was treated with pressure limited artificial ventilation and positive end expiratory pressure. After 30 hours the infant deteriorated and the chest radiograph showed air in the carotid, subclavian and aortic artery.

KEY WORDS Air embolism, artificial ventilation, idiopathic respiratory distress syndrome (IRDS).

In neonatal intensive care units respirators are frequently used for the treatment of idiopathic respiratory distress syndrome (IRDS). This disease requires the use of positive end expiratory pressures in combination with long inspiratory times and sometimes high pressure/volume in order to obtain a reasonable oxygenation and ventilation. Such therapy may produce air leaks causing interstitial emphysema, pneumothorax or pneumomediastinum. Very occasionally it may result in the injection of air directly into the systemic circulation, usually with a fatal outcome (1, 2).

normal bloodgas values, a peak pressure of 22 cmH₂O and a positive end expiratory pressure of 6 cmH₂O.

Then suddenly the baby became cyanotic with a bradycardia and deteriorating bloodgas values. A pneumothorax and the obstruction or dislodgment of the endotracheal tube were excluded. Crepitations were heard over the whole thorax and the peripheral circulation was markedly reduced. Foamy blood started to ooze from the site of the radial artery puncture.

A new chest radiograph showed pulmonary interstitial emphysema and intracardiac, intraaortic and intrahepatic gas was detected in the X ray.

Intravascular gas was also seen in both carotid arteries and both subclavian arteries (Fig. 2). The diagnosis of massive arterial air embolism was made. The baby died one hour later, having survived only 37 hours.

CASE REPORT

A male baby of 1100 g was born in a general hospital after a gestational period of 28 weeks. His Apgar score at 5 min was 1. Respiratory distress developed and immediate continuous positive airway pressure (CPAP) therapy was instituted. At 4 hours he was transferred to our specialized unit where he was intubated and connected to a Bourns MPP 00 respiratory, the peak pressure being set at 35 cmH₂O and the positive end expiratory pressure (PEEP) at 6 cmH₂O. A chest radiograph at this time showed grade IV hyaline membrane disease (Fig. 1). After 30 hours of ventilation the baby was stable with

DISCUSSION

Air embolism is being recognized as a rare but extremely serious complication of treatment of hyaline membrane disease. Only one recent case has been reported as surviving but in that case only venous air embolism was seen (3).

The mechanism resembles lung rupture in connection with free ascent during deep sea diving. This is a well known fatal complication of fast decompression resulting from overex-

6 months she had been unable to put weight on her right heel and had been walking with a limp.

Physical examination demonstrated a hyperesthetic circular area with a diameter of 2 cm on the inner side of the right heel. No swelling, cyanosis or atrophy of the foot were seen. In the lumbosacral region a vascular birth mark was noted. Deep tendon reflexes and peroneal sensibility were normal. X-rays of the calcaneus and of lumbosacral regions were normal as were thermography, strain gauge plethysmography and a bone scan of the foot.

On the suspicion of RSD a right lumbar block with 0.25% bupivacainechloride was performed with prompt and lasting effect. Repeated thermographies showed a transient marked increase in warmth of the right foot. Within a month after the block the girl was in full physical activity and during a follow-up period of 6 months no pain has recurred.

DISCUSSION

Many confusing names (including minor and major cruralgia, Sudeck atrophy and post-traumatic pain syndrome) have been applied to the symptom complex of reflex sympathetic dystrophy (RSD). Today RSD is the preferred name and the syndrome is thought to be a neurovascular disorder (2). The symptoms may occur with latency and after a trauma relatively minor in relation to the severity of the manifestations. In some instances the initiating factor is not recognized (1). The pain and hyperesthesia are usually localized to the site of injury. Sometimes the pain progresses proximally without being confined to a peripheral nerve distribution. This confuses the physicians who suggest a psychiatric component. Severe degrees of the syndrome also seem to occur more frequently in patients with certain personality traits (1, 7).

During the early stages vasomotor disturbances are often manifested by signs of vasodilatation. Later vasospastic symptoms dominate with coldness, pallor or cyanosis. Atrophic changes involving skin and nails

develop insidiously (2) but are seldom seen in paediatric patients (1). The differential diagnosis may include peripheral vascular disorder or peripheral neuritis, osteomyelitis and tenosynovitis.

Therapy for RSD should be instituted promptly to improve the possibility of permanent relief. In the early or mild stages conservative treatment with analgetics, warmth and physical therapy may be of value (1). In the younger age group a more active attitude with diagnostic blocks and in the severe stages sympathectomy is often preferred (2). Recently treatment of a child by transcutaneous nerve stimulation has been reported (6). After relief of pain active exercise is important in the process of rehabilitation.

REFERENCES

1. Bernstein H H, Singen H H, Kent J T, Kienreich H, King K, Hicks R & Hanson V. Reflex neurovascular dystrophy in childhood. *J Pediatr* 91: 211, 1978.
2. Bonica J J. Causalgia and other reflex sympathetic dystrophy. *Fostergrad Med* 53: 143, 1973.
3. Fennelich D R. Reflex sympathetic dystrophy in children. *Pediatrics* 60: 891, 1977.
4. Guntheroth W G, Chakmakjian S, Brens S C, Ricketts H J & Wiederhielm C A. Posttraumatic sympathetic dystrophy. *Am J Dis Child* 131: 411, 1971.
5. Kozin F, Hughton V & Ryan L. The reflex sympathetic dystrophy in a child. *J Pediatr* 90: 417, 1977.
6. Richlin D M, Carron H, Rowlinson J C, Susman M D, Brugher W H & Goldner R D. Reflex sympathetic dystrophy. Successful treatment by transcutaneous nerve stimulation. *J Pediatr* 93: 84, 1978.
7. Wirth F R & Rutherford R B. A civilian experience with cruralgia. *Arch Surg* 100: 633, 1930.

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CASE REPORT

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KEY WORDS Air embolism artificial ventilation idiopathic respiratory distress syndrome (IRDS)

In neonatal intensive care units respirators are frequently used for the treatment of idiopathic respiratory distress syndrome (IRDS). This disease requires the use of positive end expiratory pressures in combination with long inspiratory times and sometimes high pressure/volume in order to obtain a reasonable oxygenation and ventilation. Such therapy may produce air leaks causing interstitial emphysema, pneumothorax or pneumomediastinum. Very occasionally it may result in the injection of air directly into the systemic circulation usually with a fatal outcome (1, 2).

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A male baby of 1100 g was born in a general hospital after a gestational period of 28 weeks. His Apgar score at 5 min was 1. Respiratory distress developed and immediate continuous positive airway pressure (CPAP) therapy was instituted. At 4 hours he was transferred to our specialized unit where he was intubated and connected to a Bourns BP 00 respiratory; the peak pressure being set at 35 cmH₂O and the positive end expiratory pressure (PEEP) at 6 cmH₂O. A chest radiograph at this time showed grade IV hyaline membrane disease (Fig. 1). After 20 hours of ventilation the baby was stable with

normal bloodgas values, a peak pressure of 22 cmH₂O and a positive end expiratory pressure of 6 cmH₂O.

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DISCUSSION

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The mechanism resembles lung rupture in connection with free ascent during deep sea diving. This is a well known fatal complication of fast decompression resulting from overex-



Fig 1 Patient aged 4 hours IRDS grade IV

pansion of the lungs (4). The sequence of events is as follows. In an emergency rise to the surface during a dive with compressed air the diver develops a glottis cramp which prevents expiration. Thus air is trapped in the lungs which then blow up like balloons to much greater than their normal size (e.g. 1 volume unit of air at 5 atmospheres tends to enlarge to 5 times its size at surface atmospheric pressure).

If the lungs expand to the extent that the alveoli burst then the air is sucked into the pulmonary venous system and by way of the left ventricle to the systemic circulation. This same situation can be reproduced at a pressure of 10.6 kPa (80 mmHg) (5). Without proper treatment this condition is fatal. For the diver treatment consists of immediate recompression with higher pressure. Chabroux (6) has recommended recompression in a chamber to 15–20 atmospheres and ventilation with an oxygen/helium mixture to reduce the gas bubble size in the circulation as quickly as possible. The patient should be placed in Trendelenburg position which serves to reduce the risk of air bubbles reaching the head and causing brain damage.

Of course the premature infant is not exposed to the same high pressure situation as adult divers. Nevertheless the infant's alveoli can be overexpanded during treatment with assisted ventilation. The mechanism then

seems to follow a similar course. In neonatal medicine one should be aware of this complication and recognize it radiographically. Whenever long inspiratory times and PEEP are used with high pressure/volume and particularly when interstitial emphysema is present there is a high risk of air embolism. Signs to look for are changes in the peripheral circulation consisting of well defined migratory areas of pallor or sudden deterioration of the patient's condition without an obvious explanation.

In the event of a suspected air embolism treatment possibilities are limited. Changing the setting or even temporarily discontinuing the artificial ventilation may improve the situation. If the respirator is stopped however the infant most probably will become hypoxic in which case treatment with a pulmonary arterial vasodilator (Tolazoline) may be useful (7).

Massive air embolism should be considered as a cause of death in prematures with IRDS treated by positive pressure ventilation. It may be that the principles of treatment for air embolism in diving medicine could be re-evaluated for possible application to the care of newborns.

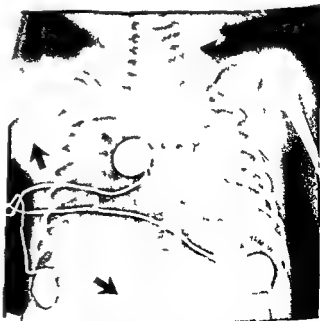


Fig 2 Patient aged 31 hours Arterial air embolism

REFERENCES

- 1 Henderson Smart D J Massive air embolism in a neonate with respiratory distress *Med J Aust* 2: 641 1975
- Siegle R L, Eyal F G & Rabinowitz J G Air embolus following pulmonary interstitial emphysema in hyaline membrane disease *Chn Radiol* 77: 77 1976
- 3 Kogutt M S Systemic air embolism secondary to respiratory therapy in the neonate: six cases including one survivor *Am J Roentgenol* 131: 4-5 1978
- 4 Kinsey J L Air embolism as a result of submarine escape training *US Armed Forces Med J* 5: 743 1954
- 5 Ehm O F *Tauchen - noch sicherer* Albert Muller Verlag Zurich 1974
- 6 Cabarro P Der Dekompressionsunfall und seine Behandlung *Munch Med Wochenschr* 108: 1557 1966
- 7 Goetzman M W, Sunshine Ph, Johnston J D, Wennberg R P, Hackel A, Merten M F, Bartolletti A L & Silverman N H Neonatal hypoxia and pulmonary vasospasm: Response to tolazoline *J Pediatrics* 89: 617 1976

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NEW BOOKS RECEIVED

- A G Coran D M Behrendt W H Weintraub & D C Lee *Surgeons of the Neonate* 777 pp illus Little Brown & Co Boston Massachusetts 1978 \$37.50 ISBN 0-316-14635-3
- F Falkner & J M Tanner (eds) *Principles and Prenatal Growth* Vol 1 In Human Growth 633 pp illus Ballière Tindall London 1978 £ 4.75 ISBN 0-700-0731-5
- F Falkner & J M Tanner (eds) *Postnatal Growth* Vol II In Human Growth 634 pp illus Ballière Tindall London 1978 £ 4.75 ISBN 0-700-0732-3
- V Dybowitz *Muscle Disorders in Childhood* Vol XVI In Major Problems in Clinical Pediatrics Saunders London and Philadelphia 1978 787 pp illus No price given ISBN 0-716-310-6
- K Roberts *Manual of Clinical Problems in Pediatrics with annotated key references* 461 pp Little Brown & Company Boston Massachusetts 1979 \$10.95 ISBN 0-316-74984-4
- J H Jonxis (ed) *Growth and Development of the Full term and Preterm Infant* The Jonxis Lecture Vol ume I Excerpta Medica Amsterdam 1978 293 pp illus \$31.00 ISBN 0-444-90050-0
- E L Kendig Jr & V Chermick (eds) *Disorders of the Respiratory Tract in Children* 3rd ed W B Saunders Company London 1115 pp illus £3.50 ISBN 0-7716-5378-7
- G Stamatoyannopoulos & A W Nienhuis (ed) *Cellular and Molecular Regulation of Hemoglobin Switching* 792 pp illus Grune & Stratton Inc 1979 \$68.50 ISBN 0-8089-1359-7
- M Muskin (ed) *Ultra Sound in Paediatrics* 337 pp illus Grune & Stratton Inc New York 1979 \$79.50 ISBN 0-8089-1117-1
- F Falkner & J M Tanner (eds) *Neurobiology and Nutrition In Human Growth* 3 606 pp illus Plenum Press New York 1979 \$37.50 ISBN 0-306-34463-7
- M Parsons *Tuberculous Meningitis* A handbook for clinicians 83 pp illus Oxford Medical Publications London 1979 £4.90 ISBN 0-19-261166-6
- L A Barnes *Advances in Paediatrics* Vol 25 510 pp Year Book Medical Publishers London 1978 \$45.25 ISBN 0-8151-0497-9
- C G Geary *Aplastic Anaemia* 249 pp illus Ballière Tindall London 1979 £1.50 ISBN 0-700-0698-X
- P H Morris Jones (ed) *Topics in Paediatrics I Haematology and Oncology* 156 pp illus Pitman Medical Tunbridge Wells England 1979 £9.50 ISBN 0-272-79540-7

BOOK REVIEWS

A. K. K. Kreutner & D. R. Hollingsworth *Adolescent obstetrics and gynecology*. Year Book Medical Publishers Inc. Chicago. London 1974. 658 pp. illus. \$40.50. ISBN 0-8151-5700-0.

This textbook, which is intended for physicians, especially obstetricians and gynecologists, students and paramedical professionals, is a useful approach to common obstetrical and gynecological problems of adolescence. Many chapters are relevant not only to teenagers but other topics have a broader application.

The first part of the book is a review of adolescent sexuality and in excellent presentation of the hypothalamic-pituitary-gonadal system. The section that follows is concerned with the maturation of this system during puberty from its quiescent state in the child to its fully operational state in the adult.

Many chapters deal with teenage pregnancies. The discussion about abortion and abortion counselling is up to date and well suited to the conditions in Sweden since the new law on abortion. The fact that the teenager alone has to decide about pregnancy is emphasized. The counsellor's role is to supply necessary information about alternatives, explain the details of any procedures and provide contraceptive information. Pregnant adolescents also require specialized prenatal care because of their age and socioeconomic circumstances. It is therefore more important to use the assistance of social workers than to give a too detailed description of the nutrition during pregnancy with dietary tables and sample menus.

Pre-eclampsia often occurs in teenage patients. The authors present a useful clinical guideline for the management of hypertensive patients. Early diagnosis and adequate treatment may prevent severe progression of the disease. Pregnant diabetic adolescents run an increased risk of pre-eclampsia. Identification of gestational diabetes is therefore important and a simple classification is presented and the therapeutic principles discussed.

Numerous studies have shown that many women—and especially teenagers—take a great many drugs during pregnancy. Since some drugs have been shown to be potentially teratogenic the authors give a comprehensive review of the most commonly ingested drugs. The book ends with detailed appendices on drugs and adolescent pregnancy.

Dysmenorrhea is the most common gynecological complaint of the adolescent. The extensive use of oral contraceptive agents has reduced its frequency but many adolescents do not like or need such hormone therapy. New therapeutic possibilities have been obtained with the different kinds of prostaglandin inhibitors.

The question of birth control and the efficacy and side effects of the various methods are briefly discussed. In contrast to the authors we in Sweden never support the opinion that intra uterine devices might be the contraceptive method of choice for the sexually active adolescent.

The chapter concerning breast disorders is very important and deals in an excellent way with all the problems for which an adolescent can consult a doctor. True gynecological neoplasms in teenagers are uncommon. Two-thirds of them develop in the ovary. Since most of the ovarian neoplasms are germ cell tumours they are unilateral, encapsulated and seldom give peritoneal metastases. This often makes conservative surgery possible and thus also preserved reproductive capacity. The increasing frequency of vaginal and cervical clear cell adenocarcinoma in adolescent girls exposed to intra uterine diethylstilbestrol prior to 10–16 weeks of gestation is discussed too. A large number of illustrations, tables and references enhance the value of the book which can be highly recommended.

Lars Svanberg

R. Mande, N. Masse & M. Manciaux (eds) *Pediatric sociale*. 2nd ed. 715 pp. Flammarion Medicine Sciences. Paris 1977. No price given. ISBN 2 257 70191 X.

The teaching of basic social paediatrics is or should be included in the ordinary curriculum of medical school. In a few countries there are special graduate or postgraduate courses in social paediatrics. In Sweden for example a one week compulsory course for specialization in paediatrics. Since the reorganization of the National Health Service in Britain the number of community paediatricians has increased rapidly and recently a university degree of Master of Medical Science in Community Paediatrics has been proposed.

In the USA a 2 year training program in social or community paediatrics is now offered at six universities. The purpose is that these paediatricians later will enter medical faculties and serve as infiltrators of socio-paediatric ideas.

The greatest experience in socio-paediatric teaching however comes from France where the Centre International de l'Enfance every year since 1950 has organized international courses in social paediatrics. At some medical schools in France there are even special chairs of social paediatrics.

Therefore it is not surprising that the first and so far the only major European text book on social paediatrics should appear in French. The first edition appeared in 1972 and now a second extended one has come out. The editors are French paediatricians well known in international socio-paediatric and educational circles. They have succeeded in making the contributors produce a very comprehensive and rich volume. The five parts of the book deal with General aspects, including demographic and biostatistical aspects of childhood. The Normal Child. The Adolescent. Social Aspects of Childhood diseases and Organization of Preventive Care. Although the experiences and points of view are mainly French there is

so much of generally applicable knowledge and information that the book is useful also for a non-French reader. It is not possible to mention all the good parts of the book. There is, for instance, a good chapter on vaccinations dealing in detail with all important childhood vaccinations and their indications and complications. In the latest edition the text on smallpox vaccination could perhaps be cut down to just one page or so.

Other parts which I have found especially valuable are those on caries, accidents and chronic diseases.

In the chapter on perinatal problems the emphasis is put on prematurity and its socio-economic causes. I think the importance of the family's socio-economic status for the ultimate outcome of the premature baby should be discussed more fully.

Curiously enough, I could not find anything at all about the newborns injured by their mothers' abuse of alcohol during pregnancy. This so-called foetal alcohol syndrome is rapidly developing into a first-rate socio-paediatric problem, not only in France where it was first described in modern times, but in all industrialized countries.

Altogether, it is a valuable and well composed book and it should be welcomed by students, nurses and paediatricians, since it gives them a full view of social paediatrics and material for new thoughts.

In one aspect, however, it is like almost all other text books: there is little criticism of conventional medical procedures, treatment and prevention. It is a pity that the knowledge and experience of sociologists and psychologists are not used more extensively. As far as I can see, almost all the authors are doctors. I also miss a critical analysis of the numerous screening procedures that are carried out in preventive paediatrics, i.e. a basic methodological discussion on evaluation principles. In my experience, this type of biostatistics is a very useful tool for a social paediatrician, whether he is reading or writing articles, and it is rarely taught in general paediatrics. By introducing a chapter on epidemiology and cutting down the chapter on French administration and organization of the book would be made still more useful internationally.

Lennart Kohler

V. Dubowitz, *Muscle disorders in children*, Vol. XVI. In: Major problems in clinical paediatrics. Saunders, London and Philadelphia 1978. 8 pp, illus. No price given. ISBN 0-716-3 10-6.

In the preface to his book, Victor Dubowitz says that the clinician's place is not only at the bedside but also

in the laboratory, in order to get a comprehensive overview of the patient and his investigations and not only try to draw conclusions from either alone.

This book gives the essence of twenty years' experience of children with muscle disorders at the bedside, combined with the clinically relevant parts of the previous laboratory book *Muscle Biopsy: A Modern Approach* by Dubowitz and M. H. Brooke (1973).

In addition to muscle disorders in the narrow sense of the word, there are chapters dealing with disorders of the lower neuron, myasthenia gravis, inflammatory myopathies, disorders with muscle contracture and joint rigidity and disorders of movement.

Professor Dubowitz has combined his broad experience as a clinician and scientist with his talent as a writer and teacher. The result is a splendid manual in the art of making the diagnosis in the entangled field of neuromuscular disorders. It will become the first choice for reference for the practising paediatrician as well as for the neurologist and orthopaedic surgeon.

O. Hansson

J. H. Jonxis (ed.), *Growth and development of the full term and premature infant*. The Jonxis Lectures, Volume 1. Excerpta Medica, Amsterdam 1978. 793 pp, illus. US \$31.00. ISBN 0-444 90050-0.

This volume contains a collection of lectures given to the Postgraduate Course on Growth and Development of the Full term and Premature Infant, held in Curaçao from 21 to 23 November 1977. The presentations are given by internationally well-known European and American scientists within the field of neonatology. The various contributions deal with topics of current interest such as regulation of growth and nutrition of the fetus and nutritional needs, energy balance and metabolism of the newborn. All individual papers are of high scientific quality and contain a rather comprehensive presentation of the topic. In the last chapter, Dr Widdowson in a very stimulating way gives comments on the various presentations.

The book can be recommended to all those who want to read a condensed presentation of current progress in some of the most important fields of neonatology.

Rolf Zetterstrom

ANNOUNCEMENT

The Fifth World Symposium on Pediatric Surgery will be held at the Acapulco Princess in Acapulco, Congress office Insurgentes held from the 15th to the 19th of October, 1980 at the Acapulco Princess, Sur 3700, Mexico 22, D.F., Mexico.